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Mechanism of Resistance, Gene Flow, and Integrated Management of Ragweeds (*Ambrosia*) in Nebraska

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MECHANISM OF RESISTANCE, GENE FLOW, AND INTEGRATED MANAGEMENT OF RAGWEEDS (*Ambrosia*) IN NEBRASKA

by

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MECHANISM OF RESISTANCE, GENE FLOW, AND INTEGRATED MANAGEMENT OF RAGWEEDS (*Ambrosia*) IN NEBRASKA

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Common ragweed (*Ambrosia artemisiifolia* L.) and giant ragweed (*Ambrosia trifida* L.) are native annual broadleaf weeds in the United States found in diverse agroecosystems, roadsides, and wastelands. They are economically important weed species in the Midwest and sources of pollen allergies. Confirmation of glyphosate-resistant (GR) common and giant ragweed in Nebraska justified the need to determine the mechanism of resistance, dispersal of resistance genes via pollen, and to develop an integrated management program. The objectives of this research were to: 1) determine the mechanism of glyphosate resistance in a common ragweed biotype from Nebraska; 2) evaluate the effect of varying growth temperatures on efficacy, absorption, and translocation of glyphosate or 2,4-D in GR and susceptible (GS) common and giant ragweed biotypes; 3) quantify the pollen-mediated gene flow (PMGF) from GR to GS giant ragweed under field conditions; and 4) evaluate the integrated management of giant ragweed with preplant tillage followed by PRE and/or POST herbicide programs in corn and soybean. Experiments were conducted to determine mutation(s), and amplification of the *EPSPS* gene (target-site mechanisms), as well as differences in uptake/translocation and the metabolism of glyphosate (non-target site mechanisms) between GR and known GS common ragweed biotypes. The results suggest that a slow rate of glyphosate absorption

and translocation likely prevents the build-up of the minimum inhibitory glyphosate concentration required at the target site, resulting in resistance to glyphosate in a common ragweed biotype from Nebraska. Experiments conducted to study the effect of temperature on the efficacy of 2,4-D or glyphosate in common and giant ragweed suggested that control improved at warm temperatures (29/17 ^{0}C d/n) compared to cooler temperatures (20/11 ^{0}C d/n) due to increased translocation in common ragweed, and increased absorption and/or translocation in giant ragweed biotypes studied. Studies on PMGF from GR to GS giant ragweed were conducted under field conditions using glyphosate resistance as a phenotypic marker. The highest frequency of gene flow (0.43 to 0.68) was detected at closer distances (< 0.5 m) and 50% reduction in gene flow occurred at < 7 m from the pollen source. Field experiments conducted to evaluate the integrated management of GR giant ragweed suggested that integration of preplant tillage would provide an alternate method for early season control of giant ragweed; however, follow-up application of herbicides are needed for season-long control in corn and soybean.

DEDICATION

This thesis is dedicated to my parents, Hajira Begum and Abdul Ahad Ganie, for all their unconditional love, support, and sacrifices to give me the best education they could.

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CHAPTER 1: INTRODUCTION AND OBJECTIVES

Introduction

Weeds have always been a component of agriculture, conflicting with human economic interests by causing crop yield losses, reduction in quality of produce, and complications in management, as well as consuming resources and time of growers, land managers, and ranchers (Baker 1991; Oerke 2006; Owen 2016). The discovery and commercialization of herbicides brought major changes in agriculture, including easy, efficient, and affordable weed control; allowed early and narrow-row planting of crops; and reducing the need for tillage and hand-weeding. These tactics increased crop yield and reduced soil erosion (Hamill et al. 2004). The discovery of phenoxyacetic herbicides in 1940s marked the real beginning of successful chemical weed control (Blackman 1948, Hamner and Tukey 1944). However, the most important breakthrough in chemical weed control was the discovery of glyphosate in the 1970s (Appleby 2005; Franz et al. 1996). Glyphosate is a systemic broad-spectrum post-emergence herbicide effective on both annual and perennial grasses and broadleaf weeds (Franz et al. 1996; Giesy et al. 2000; Sammons et al. 2007). One of glyphosate's unique features is its non-toxicity to mammals, birds, fish, or insects, since it targets a physiological pathway found only in plants and some microorganisms (Franz et al. 1996).

Glyphosate competes with phosphoenolpyruvate (PEP) to bind to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme complex, occupying the binding site almost permanently and owing to a 2,300-fold slower rate of dissociation compared to the natural association of PEP and EPSPS (Cole 1985; Devine et al. 1993). At effective doses, glyphosate inhibits the majority of EPSPS enzyme in a cell and

prevents the normal functioning of the shikimate pathway (Alarcón-Reverte et al. 2013; Funke et al. 2006). Inhibition of the EPSPS enzyme results in unregulated carbon flow through excessive production of shikimate-3-phosphate and the insufficient synthesis of aromatic amino acids (phenylalanine, tryptophan, and tyrosine) required for protein synthesis, eventually leading to plant mortality (Duke and Powles 2008; Schönbrunn et al. 2001). Due to its non-selective nature, glyphosate became widely adopted for weed control under non-crop situations, and before planting or after crop harvest in agronomic fields (Green 2009). However, the commercialization of glyphosate-resistant (GR) crops revolutionized weed control in crop production areas by expanding the use of glyphosate in soybean [*Glycine max* (L.) Merr.], canola (*Brassica napus* L.), cotton (*Gossypium hirsutum* L.), and corn (*Zea mays* L.) (James 2004). GR crops were the most rapidly adopted technology in the history of agriculture (James 2007). This situation soon resulted in over dependence on glyphosate and led to the evolution of GR weeds (Beckie 2007; Powles and Yu 2010). Glyphosate resistance has currently been reported in 35 weed species, including 16 grasses and 19 broadleaf weeds in 27 countries (Heap 2016).

In the United States, 16 weed species spread across 38 states have evolved resistance to glyphosate (Heap 2016). In Nebraska, 6 weed species, including common ragweed (*Ambrosia artemisiifolia* L.), common waterhemp (*Amaranthus rudis* Sauer), giant ragweed (*Ambrosia trifida* L.), horseweed [*Conyza canadensis* (L.) Cronq.], kochia [*Kochia scoparia* (L.) Schrad.], and Palmer amaranth (*Amaranthus palmeri* S. Wats.) have been confirmed resistant to glyphosate (Chahal et al. 2016; Jhala 2014; Sarangi et al. 2015).

***Ambrosia* species.** The genus *Ambrosia* comprises at least 40 species of weedy plants of the family *Asteraceae* (Anonymous 2006). The species comprising the genus *Ambrosia* are commonly referred to as ragweeds. Most ragweed species are native to North America, with a center of diversity located in the southwestern United States and northern Mexico (Anonymous 2006). Common and giant ragweed are the two prevalent ragweed species, well known as sources of allergenic pollen and as economically important weeds in agricultural settings. Common ragweed is normally found all over North America, but is widespread throughout the eastern United States and southeastern Canada (Jordan et al. 2007). Similarly, giant ragweed is distributed throughout the entire Midwest and East, but has become a bigger threat in row crop production systems in the eastern Corn Belt (Johnson et al. 2006; Regnier et al. 2016).

Common Ragweed Biology. Common ragweed is an erect summer annual broadleaf weed frequently found on roadsides, in wastelands, and in agronomic fields predominantly under reduced or no-till cropping systems (Bassett and Crompton 1975; Jordan et al. 2007; Saint-Louis et al. 2005). Common ragweed has been documented as a major cause of hay fever due to its prolific pollen production, which are allergenic and easily carried by wind (Fumanal et al. 2007; Rogers et al. 2006; Simard and Benoit 2011). Common ragweed grows 1 to 2 m tall with distinct male and female flowers on the same plant and produces 32,000 to 62,000 seeds plant^{-1} (Dickerson and Sweet 1971; Jordan et al. 2007). These characteristics combined with its long seed viability (~39 years) allow common ragweed to easily establish and persist as a potential dominant weed in new habitats (Bassett and Crompton 1975).

Common Ragweed Interference and Yield Loss. Common ragweed interference with crop growth results in variable yield losses depending on the density, time of emergence relative to the crop, and the type of crop infested (Jordan et al. 2007; Weaver 2001). Common ragweed is a very competitive weed in several agronomic crops, including corn and soybean (Chikoye et al. 1995; Cowbrough et al. 2003; Jordan et al. 2007); for example, Weaver (2001) reported an average yield loss of 38% in corn with a common ragweed density of ≥ 32 plants m^{-2}. Similarly, Coble et al. (1981) and Shurtleff and Coble (1985) reported 10 to 12% soybean yield loss with 2 to 4 common ragweed plants 10 m^{-1} row length. Weaver (2001) reported that common ragweed is more competitive in soybean compared to corn and caused yield losses of 65 to 70% at a density of ≥ 30 plants m^{-2}. A season-long interference of 1 common ragweed plant 1 m^{-1} row of peanut (*Arachis hypogaea* L.) resulted in 40% yield loss (Clewis et al. 2001). Therefore, management of common ragweed is imperative to reduce crop yield losses.

Herbicide Resistance in Common Ragweed. The management of common ragweed has become more complicated due to its evolution of resistance to four herbicide sites-of-action, including acetolactate synthase (ALS), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), photosystem II (PS II), and protoporphrinogen oxidase (PPO) inhibitors (Chandi et al. 2012; Heap 2016; Patzoldt et al. 2001; Rousonelos et al. 2012; Saint-Louis et al. 2005). GR common ragweed was first reported in Missouri in 2004, and subsequently in 14 other states in the United States and Ontario, Canada (Heap 2016). GR common ragweed was recently confirmed in Nebraska and it is believed that this biotype evolved independently. Therefore, this biotype provided an opportunity to evaluate the mechanism of glyphosate resistance in common ragweed, which has

remained unclear based on previous studies (Brewer and Oliver 2009; Ganie et al. 2015; Parrish 2015).

Giant Ragweed Biology. Giant ragweed, a member of the Asteraceae family, is an early emerging summer annual broadleaf weed native to North America and is also well known for its allergenic pollen grains that are a major cause of hay fever (Kil et al. 2004; Rybnicek and Jager 2001). Historically, giant ragweed was commonly found in non-crop areas, including stream banks, flood plains, rights-of-way, fence lines, and disturbed locations (Abdul-Fatih and Bazzaz 1979; Bassett and Crompton 1982). However, over the last two decades, giant ragweed has adapted to agricultural cropping systems and become a challenging weed in several agronomic crops (Johnson et al. 2006; Norsworthy et al. 2010; Steckel 2007; Vink et al. 2012). A recent survey suggested that minimum tillage, lack of crop rotation, multiple applications of the same herbicide program, the presence of giant ragweed on non-crop field edges, early and prolonged emergence, and the presence of seed-burying common earthworm (*Lumbricus terrestris* L.) are all associated with giant ragweed's increasing infestation of crop fields and difficulty in its management (Regnier et al. 2016). Giant ragweed's early emergence, rapid growth rate, large leaf size, high photosynthetic rate, and ability to germinate and survive in diverse environments (Abdul-Fatih and Bazzaz 1979; Bazzaz and Carlson 1979; Harrison et al. 2001) give it a competitive advantage in agronomic crops early in the season compared to weed species such as pigweeds that emerge relatively late (Werle et al. 2014). In addition, the evolution of a wider window of emergence over the years, particularly in arable fields, and high plasticity in plant vigor allows giant ragweed to dominate over all

other vegetation in its vicinity (Davis et al. 2013; Glettner and Stoltenberg 2015; Kelly et al. 2012; Schutte et al. 2008; 2012).

Giant Ragweed Interference and Yield Loss. Giant ragweed competition has been assessed in several agronomic crops, including corn (Harrison et al. 2001; Williams and Masiunas 2006), soybean (Baysinger and Sims 1991), and cotton (Barnett and Steckel 2013). Harrison et al. (2001) reported that giant ragweed emerging simultaneously with corn resulted in 13 and 60% yield reduction at densities of 1.7 and 13.8 plants 10 m^{-2}, respectively. Similarly, 5% loss of ear mass was reported with a giant ragweed density of 0.04 plants m^{-2} (1 plant 25 m^{-2}) in sweet corn (Williams and Masiunas 2006). Giant ragweed is even more competitive in soybean, with 1 plant m^{-2} causing 45 to 77% yield loss (Baysinger and Sims 1991; Webster et al. 1994). The critical period of weed control in soybean is 4 to 6-wk after planting (Bloomberg et al. 1982; Coble et al. 1981; Williams and Hayes 1984); however, to avoid soybean yield losses due to giant ragweed interference, its critical period extends from 8 to 10-wk after soybean emergence (WAE) (Baysinger and Sims 1991).

Herbicide Resistance in Giant Ragweed. Giant ragweed is an allogamous species with a wind-pollinated nature, has great genetic diversity and potential for rapid evolution of herbicide resistance (Bassett and Crompton 1982; Johnson et al. 2006). Giant ragweed has evolved resistance to ALS-inhibitors in 5 states and to glyphosate in 13 states in the United States and in Ontario, Canada (Heap 2016). In addition, four biotypes of giant ragweed with multiple-resistance to both ALS-inhibitors and glyphosate have been reported, three from the United States and one from Canada. In contrast, Regnier et al. (2016) reported that a survey on giant ragweed distribution conducted in 15 states

indicated resistance to ALS-inhibitors, to glyphosate and to both modes of action is present in 13, 14, and 12 states respectively. The authors further concluded that respondents to the survey perceive more area to be affected by resistance to ALS-inhibitors and resistance to both ALS-inhibitors and glyphosate.

Objectives

GR common and giant ragweed have been confirmed in Nebraska, making it imperative to address the information gaps that exist under local agro-ecological conditions to understand the mechanism of resistance, gene flow, and integrated management strategies for these species. Because the precise mechanism of glyphosate resistance in *Ambrosia* species is not clear, a study was conducted to determine the mechanism of glyphosate resistance in a GR common ragweed biotype from Nebraska. Common and giant ragweed are early emerging weed species and their preplant management is essential to allow crop planting under weed-free conditions. However, variation in early-season temperature might influence the efficacy of commonly used preplant herbicides such as 2,4-D or glyphosate for control of ragweeds. Thus, it is important to study the effect of varying growth temperatures on the efficacy of 2,4-D or glyphosate in common and giant ragweed and how varying temperaturs affect their absorption and translocation. In addition, there is no information available on the dissemination of glyphosate resistance in giant ragweed, and to fill this information gap, pollen-mediated gene flow from GR to GS giant ragweed was evaluated under field conditions. Over dependence on herbicides with the same mode of action have enhanced the evolution of herbicide resistant weeds, and to deal with this situation, the diversification of weed management strategies has

become indispensable. Integrated management of GR giant ragweed was evaluated using preplant tillage along with PRE followed by POST herbicides in both corn and soybean.

The objectives of this research were:

1) To evaluate the physiological and molecular mechanism of the GR common ragweed biotype from Nebraska;

2) To study the effect of varying growth temperatures on the efficacy, absorption, and translocation of 2,4-D or glyphosate in common and giant ragweed;

3) To quantify pollen-mediated gene flow from GR to GS giant ragweed under field conditions; and

4) To determine integrated management of GR giant ragweed with preplant tillage and PRE followed by POST herbicide programs in corn and soybean.

The hypotheses of this research were:

1) Reduced absorption and translocation will be the mechanism of resistance in GR common ragweed biotype from Nebraska;

2) Lower temperature will decrease the efficacy of preplant herbicides including 2,4-D or glyphosate on common and giant ragweed control;

3) Pollen-mediated gene flow from GR to GS giant ragweed will be involved in the dispersal of glyphosate resistance under field conditions; and

4) Preplant tillage and PRE followed by (fb) POST herbicide programs will provide a better giant ragweed control compared to PRE fb POST herbicides in corn and soybean.

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CHAPTER 2: INVESTIGATION OF THE MECHANISM OF GLYPHOSATE RESISTANCE IN A COMMON RAGWEED (*Ambrosia artemisiifolia*) BIOTYPE FROM NEBRASKA

Abstract

Common ragweed is a difficult-to-control weed in the Midwestern United States due to the evolution of resistance to multiple herbicides, including glyphosate. Recently, a common ragweed biotype with 19-fold glyphosate resistance was confirmed in Nebraska. The objective of this study was to determine the mechanism of glyphosate resistance in a common ragweed biotype from Nebraska. Mutation(s), and amplification of the *EPSPS* gene (target-site mechanisms), as well as differences in uptake/translocation and the metabolism of glyphosate (non-target site mechanisms) between glyphosate-resistant (GR) and known glyphosate-susceptible (GS) common ragweed biotypes were determined. A lower amount of shikimate was accumulated in the GR (< 60 μg ml^{-1}) compared to the GS (≥ 80 μg ml^{-1}) biotype at all glyphosate concentrations (50, 100, 150, and 250 μM) tested. Sequencing of the conserved region of the *EPSPS* gene revealed no mutations at the Thr_{102} or Pro_{106} residues, known to confer resistance to glyphosate. Similarly, no variation in *EPSPS* copy number was detected between GR and GS biotypes. No metabolism of glyphosate was detected to explain the mechanism of resistance. Further analysis using the rectangular hyperbolic model predicted a slower rate of absorption and translocation of glyphosate in the GR compared to the GS biotype, though more research is needed. These results suggest that a slow rate of glyphosate absorption and translocation likely prevents the build-up of the minimum inhibitory

concentration of glyphosate required at the target site, resulting in resistance to glyphosate in a common ragweed biotype from Nebraska. The outcome of this study offers a new direction for further investigation of the precise mechanism of glyphosate resistance in this biotype.

Introduction

Common ragweed, a summer annual broadleaf weed, is found in diverse agroecosystems, wastelands, and roadsides (Bassett and Crompton 1975; Jordan et al. 2007; Saint-Louis et al. 2005). Common ragweed is a natural colonizer, producing 32,000 to 62,000 seeds plant^{-1} when permitted to grow for the entire season without competition from crop plants (Dickerson and Sweet 1971; Friedman and Barrett 2008; Jordan et al. 2007). Common ragweed seeds usually germinate on or near the soil surface, preferably within 5 cm depth (Jordan et al. 2007; Stoller and Wax 1973). Small seed size, specific requirements of light and temperature for germination, and a preference for undisturbed habitats has made common ragweed a predominant weed in reduced or no-till cropping systems in the Midwestern United States (Jordan et al. 2007). High selection pressure due to exclusive dependence on chemical weed control in no-till cropping systems combined with a wide genetic diversity has resulted in the evolution of resistance to several herbicide sites-of-action in common ragweed (Brewer and Oliver 2009; Duke and Powles 2009; Rousonelos et al. 2012; Saint-Louis et al. 2005; Schultz et al. 2000). Glyphosate-resistant (GR) common ragweed was first reported in Missouri in 2004, and subsequently in 14 other states in the United States (Alabama, Arkansas, Indiana, Kansas, Kentucky, Minnesota, Mississippi, Nebraska, New Jersey, North Carolina, North Dakota, Ohio, Pennsylvania, and South Dakota) and in Ontario, Canada (Heap 2016). Additionally,

common ragweed biotypes resistant to acetolactate synthase (ALS), photosystem II (PS II), and protoporphrinogen oxidase (PPO) inhibitors have been reported (Chandi et al. 2012; Heap 2016; Patzoldt et al. 2001; Rousonelos et al. 2012; Saint-Louis et al. 2005).

Glyphosate is a POST-applied, non-selective herbicide with the ability to control a wide spectrum of broadleaf, grass, and perennial weeds, and its lack of residual activity, low cost, and relatively safe environmental profiles (including its non-toxicity to mammals, birds, fish or insects) made it the most widely used herbicide throughout the world (Dill et al. 2010; Duke and Powles 2008). Glyphosate competes with phosphoenolpyruvate to irreversibly bind to 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and inhibits normal function in the shikimate pathway (Alarcón-Reverte et al. 2013; Funke et al. 2006). Inhibition of the EPSPS enzyme results in unregulated carbon flow through excessive production of shikimate-3-phosphate and the insufficient synthesis of aromatic amino acids (phenylalanine, tryptophan, and tyrosine) required for protein synthesis, eventually leading to plant mortality (Duke and Powles 2008; Schönbrunn et al. 2001).

The commercialization and rapid adoption of GR crops encouraged reliance on glyphosate for broad-spectrum weed control that resulted in the evolution of GR weeds (Duke and Powles 2009; Powles 2008; Powles and Yu 2010). As of 2016, glyphosate resistance has been confirmed in 36 weed species worldwide, including 16 species in the United States (Heap 2016). Previous studies have revealed that glyphosate resistance is conferred due to one or a combination of several mechanisms, including target-site mutations (Powles and Yu 2010), amplification/elevated expression of the *EPSPS* gene (target-site mechanisms) (Gaines et al. 2010), active vacuolar sequestration (Ge et al.

2010), limited cellular uptake, restricted translocation (Lorraine-Colwill 2002), and rapid necrosis response (non-target site mechanisms) (Sammons and Gaines 2014; Van Horn and Westra 2014).

Target-site mutations cause conformational changes in the structure of the EPSPS enzyme and decrease its affinity for glyphosate while maintaining the normal function of the enzyme (Funke et al. 2009). Target-site mutations with the substitution of proline by serine, alanine, threonine, or leucine at position 106 (corresponding to the *Arabidopsis EPSPS* sequence) of *EPSPS* have been reported in GR biotypes of goosegrass [*Eleusine indica* (L.) Gaertn.] (Baerson et al. 2002; Ng et al. 2003), Italian ryegrass (*Lolium multiflorum* Lam.) (Perez-Jones 2007), junglerice [*Echinochloa colona* (L.) Link] (Alarcón-Reverte et al. 2013), rigid ryegrass (*Lolium rigidum* Gaudin) (Bostamam et al. 2012; Kaundun et al. 2011; Simarmata et al. 2008), sourgrass [*Digitaria insularis* (L.) Mez ex Ekman] (Carvalho et al. 2012), tall waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] (Bell et al. 2013; Nandula et al. 2013), and recently a double mutation with Pro106 to Ser and Thr102 to Ile substitutions conferring a high level of glyphosate resistance was reported in goosegrass (Yu et al. 2015). Alternatively, gene amplification or elevated *EPSPS* expression leads to an increase in the level of the EPSPS enzyme—as reported first in Palmer amaranth (*Amarannnthus palmeri* S Wats.), which can also confer resistance to glyphosate even though the EPSPS enzyme remains susceptible to glyphosate (Gaines et al. 2010; 2011). Glyphosate resistance because of *EPSPS* gene amplification was also reported in Italian ryegrass (Salas et al. 2012), kochia [*Kochia scoparia* (L.) Schard.] (Kumar et al. 2015; Wiersma et al. 2015), spiny amaranth

[*Amaranthus spinosus* (L.)] (Nandula et al. 2014), and tall waterhemp (Chatham et al. 2015; Lorentz et al. 2014; Sarangi 2016; Tranel et al. 2011).

In contrast, non-target site mechanisms restrict the accumulation of glyphosate at the critical/toxic concentrations required to inhibit the EPSPS enzyme in the chloroplast (Powles and Yu 2010; Sammons and Gaines 2014). Non-target-site mechanisms such as reduced absorption and/or translocation of glyphosate are considered the most commonly occurring mechanisms in GR weed species (Powles and Yu 2010; Shaner 2009). Altered translocation has been reported in GR hairy fleabane [*Conyza bonariensis* (L.) Cronq.] (Dinelli 2008), horseweed, and *Lolium* species (Ge et al. 2010; 2012). In addition, several weed species with more than one mechanism of glyphosate resistance in the same population have been reported. For example, González-Torralva et al. (2012) reported impaired glyphosate translocation and glyphosate metabolism into glyoxylate, sarcosine, and aminomethylphosphonic acid (AMPA) as the mechanism of glyphosate resistance in a horseweed population from Spain.

Despite some earlier attempts, the precise mechanism of glyphosate resistance in common ragweed is unknown. Brewer and Oliver (2009) reported that a target-site mutation, reduced absorption, and translocation do not contribute to the mechanism of resistance in GR common ragweed biotypes from Arkansas. Similarly, Parrish (2015) did not find conclusive results to explain the mechanism of glyphosate resistance in a common ragweed biotype from Ohio, but suggested the presence of multiple mechanisms within the same biotype. Likewise, the mechanism of glyphosate resistance in giant ragweed (*Ambrosia trifida* L.), a closely related species to common ragweed, is also unclear, though after evaluating all possible mechanisms, Van Horn and Westra (2016)

ruled out the possibility of mutation at Pro_{106} or increased *EPSPS* activity and suggested that an altered translocation might be conferring the resistance. Glyphosate-resistant common ragweed confirmed for the first time in Nebraska provided an opportunity to evaluate the mechanism of glyphosate resistance in common ragweed that remains unclear based on previous studies. Therefore, the objectives of this study were to determine the mechanisms of glyphosate resistance in a common ragweed biotype from Nebraska.

Materials and Methods

Plant Material and Growth Conditions. A common ragweed biotype from Gage County (40.44°N, 96.62°W), NE with a 19-fold level of glyphosate resistance (Ganie et al. 2015) was investigated to determine the mechanism of resistance in this study. Seeds of a known GS common ragweed biotype collected from a field near Clay Center, NE (40.52°N, 98.05°W) were used for comparison with the GR common ragweed biotype in all experiments. Glyphosate susceptibility or resistance was confirmed for the plants used for DNA extraction by spraying vegetative clones raised by planting growing tips treated with rooting power under locally made high humidity chambers. The clones of GR and GS common ragweed were treated with 1,260 g ae ha^{-1} of glyphosate (Touchdown HiTech®, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC). Common ragweed seeds were germinated in plastic trays containing potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, Quebec, Canada) and after the appearance of the first true leaves, uniform-sized seedlings were transplanted to square plastic pots (8 cm × 8 cm × 9 cm) containing a 3:1 mixture of potting mix to soil. Plants were supplied with adequate water and nutrients as needed. Uniform growth conditions

were maintained for the experiments with day/night temperatures of 25 ± 2/18 ± 3 C, and sodium halide lamps (250 µmol $m^{-2}s^{-1}$) were used as a supplemental light source to ensure a 15-h photoperiod.

Shikimate Assay. Common ragweed plants were grown as described above. Eight plants from each biotype were used for the shikimate assay following the protocol described by Shaner et al. (2005). Leaf discs (5-mm-diam) were excised from a fully expanded top leaf on each plant and placed into a single well of a 96-well flat-bottomed microtiter plate containing 0, 50, 100, 150, and 250 µM glyphosate and a 10 mM ammonium phosphate buffer (pH 7). The plates were incubated under fluorescent light at 560 µmol $m^{-2}s^{-1}$ for 16 h. After the incubation period, 25 µl of 0.05 M HCl was added to each well and the samples were freeze-thawed through two cycles of -20 C for 90 min followed by 60 C for 20 min until the green color of the leaf tissues had faded away (Nguyen 2015; Shaner et al 2005). From each well, 25 µl of the solution was transferred to fresh microtiter plates to determine shikimate levels. Shikimic acid was added to empty wells at 1, 2.5, 5, 10, 25, 50, and 100 µM concentrations as standards. A mixture of 0.25% (w/v) periodic acid (H_5IO_6) and 0.25% (w/v) sodium m-periodate ($NaIO_4$) was added to wells of both extract and standard shikimic acid at a volume of 100 µl per well. The samples were incubated at room temperature for 60 min, after which a freshly made quench buffer (a mixture of 0.6 M NaOH and 0.22 M Na_2SO_3) was added (100 µl per well) to halt the reaction.

Shikimate accumulation was determined at 380 nm on a 96-well plate reader (BioTek™ Synergy™ 2 multi-mode microplate reader, Winooski, VT). A shikimate standard curve was developed to quantify shikimate accumulation (ng shikimate $µl^{-1}$) in the experimental samples (Shaner et al. 2005). The experiment was conducted in a

completely randomized design with four replicates and the experiment was repeated three times. The shikimate data were subjected to ANOVA in SAS version 9.3 (SAS Institute Inc, Cary, NC) using the PROC GLIMMIX procedure to test for treatment by experiment interaction. Shikimate accumulation data were regressed over glyphosate doses using a two-parameter asymptotic regression model in GraphPad Prism 6 (GraphPad Software, Inc., Avenida de la Playa, La Jolla, CA):

$$y = A_{max}\left\{1 - \exp[(log0.1) \times \left(\frac{x}{e}\right)]\right\} \quad [1]$$

where y is the shikimate accumulation (µg ml^{-1}) in response to glyphosate dose (µM), A_{max} is the upper limit or maximum amount of shikimate accumulation at a higher glyphosate dose, x is the glyphosate dose, and e is the dose required to reach 90% of the maximum shikimate accumulation.

EPSPS Gene Sequencing. Ten common ragweed plants each of the GS and GR biotypes were sampled, and the experiment was repeated twice. A 100-mg sample of young leaf tissue was harvested, flash frozen, and ground to a fine powder in liquid nitrogen (-195.79 C) using a pre-chilled mortar and pestle. The genomic DNA (gDNA) was extracted using DNAzol® following the manufacturer's protocol (Invitrogen™, Thermo Fisher Scientific Inc.). Quality and the concentration of gDNA was determined by using gel electrophoresis (0.8% agarose) and a NanoDrop (ND-1000) spectrophotometer (Thermo Fisher Scientific, Waltham, MA). A polymerase chain reaction (PCR) was performed on gDNA in a T100 thermal cycler (BioRad Inc., Hercules, CA) to amplify the conserved region of *EPSPS* covering Pro_{106} and Thr_{102} codons with the primers used by Gaines et al. (2010) (Table 2.1). Each 50 µl reaction volume consisted of 25 µl of PCR master mix, 5 µl of forward primer (5 µM), 5 µl of reverse primer (5 µM), 3 µl of gDNA

template (15 ng μl^{-1}), and 12 µl of nuclease-free water. The thermocycler conditions for PCR were initial denaturation at 95 C for 3 min followed by 40 cycles of denaturation at 95 C for 30 s, primer annealing at 56 C for 30 s, product extension at 72 C for 1 min, and a final extension cycle at 72 C for 5 min. The PCR products were run on 1% agarose gel stained with ethidium bromide using 500 bp and 100 bp markers to confirm amplicon size (195 bp). PCR products were purified using a GeneJet PCR purification kit (Thermo Fisher Scientific Inc.) and quantified using a Nanodrop Spectrophotometer. About 15 µl of the purified PCR product (25 ng μl^{-1}) was sequenced at the Kansas State University sequencing facility using an ABI 3730 DNA analyzer (Applied Biosystems, Thermo Fisher Scientific Inc.). MultAlin software was utilized to align and analyze the *EPSPS* nucleotide sequences for the presence of any known target-site mutation(s) reported to confer glyphosate resistance (Corpet 1988).

Relative EPSPS Genomic Copy Number. The genomic DNA of eight GR and four GS plants was used for real-time quantitative PCR (qPCR) to determine the *EPSPS* gene copy number using *β-tubulin* as a reference gene for normalization (Godar 2015). The *EPSPS* gene copies were measured relative to the calibrator sample (a known GS biotype). The qPCR was performed using a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific Inc.), and the primer sequences used in qPCR are presented in Table 2.1. Additional common ragweed-specific qPCR primers were designed based on the *EPSPS* sequence obtained in this study and used to confirm the results obtained with the previous set of primers (Table 2.1). The common ragweed-specific qPCR primers were designed using OligoAnalyzer 3.1 (IDT SciTools, 2014; Integrated DNA Technologies, Inc., Coralville, IA). The reaction mix for qPCR consisted of 8 µl of

SYBR Green mastermix (Bio-Rad), 2 µl each of forward and reverse primers (5 µM), and 2 µl of gDNA (15 ng μl^{-1}) to bring the total reaction volume to 14 µl. The qPCR thermal specifications were 95 C for 15 min, 40 cycles of 95 C for 30 s, and 60 C for 1 min, followed by a melt-curve analysis. The melt-curve profile was generated to determine the specificity of the qPCR reaction. The relative gene copy number was determined by using the $2^{\Delta CT}$ method, where CT is the threshold cycle and ΔCT is $CT_{\text{Target gene (}\textit{EPSPS}\text{)}}$-$CT_{\text{Reference gene (}\textit{\beta-tubulin}\text{)}}$ (Gaines et al. 2010).

Absorption and Translocation of Glyphosate. Seeds of common ragweed biotypes were germinated in plastic trays containing potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, Quebec, Canada), and uniform-sized plants were transplanted at the two-leaf stage and shifted to a growth chamber at 4 d after transplanting. The plants were maintained at 28/22 (± 2) C day/night temperatures, 75% (± 4) relative humidity, and a 15 h photoperiod. Eight to 10 cm tall plants were selected for absorption and translocation experiments and sprayed with 1,260 g ae ha^{-1}, rate of glyphosate after covering a fully expanded young leaf with plastic wrap (Saran™ Premium Wrap, Racine, WI). The plastic wrap was carefully removed after the spray and the leaf was marked. Within an hour of glyphosate spray, ten 1 µl droplets of ^{14}C-glyphosate (0.33 kBq μl^{-1}) (PerkinElmer Inc. 549 Albany Street, Boston, MA) were applied to the upper surface of the marked leaf using a micro-applicator. The ^{14}C-glyphosate solution was prepared by mixing ^{14}C-glyphosate with a commercial formulation of glyphosate (Touchdown HiTech®, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC) and distilled water to achieve a final concentration equivalent to 1,260 g ae ha^{-1}. Plants were dissected at 8, 24, 48, 72, 96, 120, and 168 h

after treatment (HAT) into treated leaf (TL), tissues above treated leaf (ATL), tissues below treated leaf (BTL), and roots. The treated leaf was cut at the point of attachment to the stem and the roots were washed over wire mesh to remove soil. Treated leaves were rinsed twice in a 20 ml scintillation vial containing 5 ml wash solution (1:1 v/v mixture of methanol and deionized water and 0.05% tween-20) for 1 min to remove the unabsorbed herbicide from the surface of the treated leaf. The leaf rinse was mixed with 15 ml of scintillation cocktail, and the radioactivity was determined by using liquid scintillation spectrometry (LSS) (Tricarb 2100 TR Liquid Scintillation Analyzer; Packard Instrument Co., Meriden, CT). Plant sections were dried at 55 C for 72 h and combusted in a biological oxidizer (OX-501, RJ Harvey Instrument, NY) to recover ^{14}C labelled glyphosate in a proprietary ^{14}C-trapping scintillation cocktail and radio-assayed using LSS. Herbicide absorption and translocation were calculated as Godar et al. (2015):

$$\%\ Absorption = \left(\frac{Total\ radioactivity\ applied - Radioactvity\ recovered\ in\ wash\ solution}{Total\ radioactivity\ applied} \times 100\right) \quad [2]$$

$$\%Translocation = 100 - \%\ Radioactivity\ in\ treated\ leaf \quad [3]$$

where $\%\ Radioactivity\ in\ treated\ leaf = \frac{Radioactivity\ recovered\ in\ treated\ leaf}{Radioactivity\ absorbed} \times 100$

The experiments were arranged in a randomized complete block design by blocking to overcome variability due to plant size with four replications and the experiment was repeated twice. Data from absorption and translocation experiments were subjected to ANOVA in SAS version 9.3 (SAS Institute Inc, Cary, NC) using the PROC GLIMMIX procedure. Common ragweed biotypes (GR and GS), harvest time, and their interactions were considered fixed effects, and the experimental runs were considered as random effects. However, significant biotype by time interaction for absorption and translocation warranted further exploration of the data using the regression analysis to

include the time structure of the observations (Burke et al. 2007; Grangeot et al. 2006; Kniss et al. 2011; Nandula and Vencill 2015). A rectangular hyperbolic model was selected from the models reported in the literature based on Akaike's Information Criterion (AIC) to explain the relationship of the measured responses over time (Burke et al. 2007; Kniss et al. 2011). The rectangular hyperbolic model was fit to the data using GraphPad Prism 6 (GraphPad Software, Inc., Avenida de la Playa, La Jolla, CA 92037):

$$y = \frac{(A_{max} \times t)}{(0.11 \times t_{90} + t)} \qquad [4]$$

where y is the percentage of the applied ^{14}C-glyphosate absorbed or translocated in the plant, A_{max} is the asymptote or maximum absorption or translocation expressed as the percent applied, t is the time (h) after herbicide application, and t_{90} is the time required for 90% of the maximum absorption or translocation to occur.

Metabolism of Glyphosate. GR and GS common ragweed plants (6- to 8 cm-tall) were selected and treated with ^{14}C-glyphosate as described above in the absorption and translocation study, the only difference being that fifteen 1 µl droplets of ^{14}C-glyphosate (0.33 kBq µl^{-1}) were applied to facilitate the recovery and easy detection of radioactivity. At 48 and 96 HAT, the treated leaves were harvested and rinsed as described in the absorption and translocation study. Whole-plant tissues including the washed treated leaf were then frozen in liquid nitrogen and homogenized with a pre-chilled mortar and pestle. ^{14}C-glyphosate and its metabolites were extracted with 15 ml of 25% acetonitrile at 20 C for 30 min, and samples were centrifuged at 6,500 rpm (5,000 g) for 25 min. Supernatant was concentrated at 50 C for 2 to 4 h depending on the rate of evaporation until a final volume of 600 µl was reached with a rotary evaporator (Centrivap, Labconco, Kansas City, MO). About 600 µl of the extract was transferred to a 1.5 ml micro centrifuge tube

and centrifuged at a high speed (13,000 rpm/10,000 g) for 20 min. Radioactivity in each sample was measured by LSS before high-performance liquid chromatography (HPLC) analysis, and the samples were normalized to 60 dpm μl^{-1} (amount of ^{14}C compounds) by diluting the samples with 25% acetonitrile (55).

Total extractable radioactivity in 50 µl of the samples was resolved into parent glyphosate and its polar metabolites by reverse-phase HPLC (System Gold, Beckman Coulter, Pasadena, CA, USA). Reverse-phase HPLC was performed with a Zorbax SAX Column (4.6 × 250 mm, 5 µm particle size; Agilent Technologies, Santa Clara, CA) at a flow rate of 1 ml min^{-1} with eluent A (1 to 5 mM KH_2PO_4, pH = 2) and eluent B (1 to 100 mM KH_2PO_4, pH = 2) (Pollard et al. 2004). The elution profile was programmed as 0% B for 1 min and 0 to 100% B in 12 min. In between injections, solvent B was used to wash, and solvent A to re-equilibrate the columns. The retention time of the parent compound, ^{14}C-glyphosate, was determined by injecting 50 µl of 60 dpm μl^{-1} ^{14}C-glyphosate diluted with 25% acetonitrile. The parent compound was detected by a radio flow detector and displayed a retention time of 12.65 min. The treatments were replicated four times and the experiment was repeated twice.

Results and Discussion

Shikimate Accumulation. Treatment-by-experiment interaction for shikimate accumulation was not significant; therefore, data were combined over three experiments. Both GR and GS common ragweed biotypes showed shikimate accumulation in response to glyphosate; however, higher shikimate accumulation was observed in the GS biotype at all glyphosate concentrations (Figure 2.1). The estimated parameters of the asymptotic regression model for shikimate accumulation to glyphosate concentration were $y =$

$60\left\{1-\exp[(log0.1)\times\left(\frac{x}{72}\right)]\right\}$ with a root mean square error (RMSE) of 8.8 for the GR biotype and $y=115\left\{1-\exp[(log0.1)\times\left(\frac{x}{32}\right)]\right\}$ with a RMSE of 4.3 for the GS biotype, where y represents the shikimate accumulation (µg ml^{-1}) and x represents the glyphosate concentration (µM). The model predicted maximum shikimate accumulation of 115 µg ml^{-1} in the GS biotype compared to 60.5 µg ml^{-1} in the GR biotype at a glyphosate concentration of 250 µM. The model also predicted that the glyphosate concentration required to reach 90% of the maximum shikimate accumulation in the GS biotype was 32 µM, compared to 72 µM in the GR biotype. Similarly, Pollard et al. (2004) reported 3-fold more shikimate accumulation in GS common ragweed from Missouri compared to a GR biotype. Norsworthy et al. (2010) also reported 3.3- to 3.8-fold more shikimate accumulation in GS giant ragweed compared to the GR biotype. In contrast, Brewer and Oliver (2009) reported an identical pattern of shikimate accumulation in GR and GS biotypes of common ragweed from Arkansas, though shikimate accumulation stabilized in the GR biotype at 3 DAT but continued to increase in the GS biotype. Nol et al. (2012) reported less shikimate accumulation in a GR compared to a GS biotype of horseweed from Crete Island, Greece.

The accumulation of shikimate in the GR biotype of common ragweed provided evidence about the sensitivity of *EPSPS* to glyphosate. In addition, a rise in shikimate levels with increasing glyphosate concentrations supports the possibility of non-target site mechanisms as the potential cause of resistance, because at higher concentrations more glyphosate likely concentrates at the target site, leading to an increase in shikimate accumulation. Based on the results of the shikimate assays, non-target site mechanisms have been confirmed for glyphosate-resistance in horseweed (Koger and Reddy 2005;

Nol et al. 2012) and giant ragweed (Norsworthy et al. 2010). However, increases in shikimate accumulation due to increasing glyphosate concentration was observed in Palmer amaranth with elevated *EPSPS* copy number as the resistance mechanism (Mithila Jugulam, unpublished data).

Target-Site Mutation. The region of *EPSPS* about 145-bp long covering the Thr_{102} and Pro_{106} residues was sequenced to identify the point mutations (Pro_{106}Ser and Thr_{102}Ile) known to confer glyphosate resistance. There were no differences in the *EPSPS* sequence of the GR and GS common ragweed biotypes (Table 2.2). These results suggest that glyphosate resistance in common ragweed from Nebraska did not evolve as a result of mutations in the *EPSPS* gene. Similarly, the role of the altered *EPSPS* was ruled out as the likely mechanism of glyphosate resistance in a common ragweed biotype from Ohio (Parrish 2015), giant ragweed biotypes from across the United States (Van Horn and Westra 2016), and kochia from Montana, Kansas, and Colorado (Godar et al. 2015; Kumar et al. 2015; Wiersma et al. 2015).

Relative EPSPS Genomic Copy Number. The qPCR results exhibited no differences in the *EPSPS* gene copy number between the GR and the GS biotypes. The relative *EPSPS* gene copy number varied from 1 to 2 (Figure 2.2) and no amplification of *EPSPS* was observed in the GR biotype to explain the basis of glyphosate resistance. Similar to the results of this study, *EPSPS* gene amplification was not the mechanism of glyphosate resistance in tall waterhemp from Mississippi (Nandula et al. 2013) or giant ragweed biotypes from across the United States (Van Horn and Westra 2016). In contrast, Parish (2015) suggested increased relative *EPSPS* gene copy number as one of the mechanisms contributing to glyphosate resistance in a common ragweed biotype from Ohio. Several

studies also revealed a high number of relative *EPSPS* gene copies as the primary mechanism of glyphosate resistance in Italian ryegrass (25 copies) (Salas et al. 2012), kochia (4 to 10 copies) (Kumar et al. 2015; Wiersma et al. 2015), Palmer amaranth (5 to >160 copies) (Gaines et al. 2010), spiny amaranth (33 to 37 copies) (Nandula et al. 2014), and tall waterhemp (5 copies) (Lorentz et al. 2014; Sarangi 2016; Tranel et al. 2011).

Absorption and Translocation of Glyphosate. Treatment-by-experiment interaction for glyphosate absorption and translocation was not significant; therefore, data were pooled over the two experiments. Recovery of ^{14}C-glyphosate was similar in GR and GS biotypes across the experiments. More than 80% ^{14}C-glyphosate was recovered at 8 HAT, followed by 69 to 70% at 24, 48, 72, and 96 HAT, and 60 to 65% at 168 HAT. A similar pattern of ^{14}C-glyphosate recovery was reported in a common ragweed biotype from Arkansas with ≥ 80% recovery at 6 HAT and 68 to 79% recovery at 48 HAT (Brewer and Oliver 2009).

Total absorption expressed as the percent of applied ^{14}C-glyphosate was similar in GR (82%) and GS (84%) biotypes (Figure 2.3A). Brewer and Oliver (2009) reported that mean absorption varied from 38 to 80% of the applied ^{14}C-glyphosate at 24 HAT in common ragweed biotypes from Arkansas without any differences between the GR and GS biotypes. Similarly, Nandula et al. (2015) reported the same pattern of glyphosate absorption in the GR and GS biotypes of giant ragweed with 17 to 18 h required to complete 50% of absorption. However, in this study, the rectangular hyperbolic model predicted a rapid absorption of glyphosate in the GS common ragweed biotype compared to the GR biotype. The time required for 90% absorption of ^{14}C-glyphosate to occur in the GS plants was 22 HAT compared to 31 HAT in the GR plants (Table 2.3). In contrast,

Grangeot et al. (2006) reported 100% uptake of ^{14}C-glyphosate in a common ragweed biotype at 24 h with 50% absorption completed within 3 HAT.

The biotype by time of harvest interaction and the main effect of the time of harvest of plant samples was significant with respect to the translocation of ^{14}C-glyphosate (data not shown). The rectangular hyperbolic model predicted 73 and 84% translocation of the absorbed ^{14}C-glyphosate in the GS and GR biotypes, respectively (Table 2.3). Reduced translocation in susceptible plants possibly occurred due to the effect of glyphosate on the photosynthesis and carbon export processes in the source leaves, along with glyphosate-induced inhibition of the assimilate metabolism in sink tissues (Geiger and Bestman 1990; Geiger et al. 1999). The GS biotype showed a rapid rate of translocation with 90% of the total translocation completed within 26 HAT compared to 69 HAT required for the GR biotype (Table 2.3). Geiger et al. (1999) observed that export of glyphosate ceased by 10 HAT in susceptible sugar beet (*Beta vulgaris*) plants while it continued in the GR plants up to a period of 30 HAT. Similarly, translocation continued for 2 to 3 d after treatment (DAT) in conventional corn (*Zea mays* L.) compared to 5 DAT in GR corn (Hetherington et al. 1999). ^{14}C-glyphosate translocated to tissues above the treated leaf varied from 13 to 14% of the absorbed and did not differ between the two biotypes (Table 2.3). Similarly, ^{14}C-glyphosate translocated to above-ground tissues below the treated leaf and to the roots did not differ between GR and GS biotypes (Figure 2.4C and 2.4D). Though, the regression parameters suggest more time was required to complete 90% translocation to different plant sections, including tissues above or below the treated leaf in GR compared to GS common ragweed (Figure 2.4B, 2.4C, and Table 2.3); however, additional evidences related to the

sub-cellular distribution of glyphosate are needed to reach a conclusion about the exact mechanism. Feng et al. (1999) reported delayed and decreased leaf loading and export of glyphosate in the treated leaf of GR horseweed compared to the GS treated leaf. Similarly, Nandula et al. (2015) reported a higher rate of translocation in the GS giant ragweed biotype compared to the GR biotype. Additionally, the non-linear regression parameters indicated that 50% translocation occurred within 21.8 HAT in the GR biotype compared to 9.9 HAT in the GS biotype, and results were confirmed by phosphor-imaging (Nandula et al. 2015).

Glyphosate Metabolism. The results of reverse-phase HPLC demonstrated that no metabolism of glyphosate occurred in either the GR or GS biotypes at 48 or 96 HAT (Figure 2.5). These results indicated that metabolic deactivation or decomposition does not contribute to glyphosate resistance in common ragweed from Nebraska. These results are consistent with previous reports that demonstrated metabolic deactivation was not the mechanism of glyphosate resistance in goosegrass (Tran et al. 1999), horseweed (Dinelli et al. 2006; Feng et al. 2004), or rigid ryegrass (Feng et al. 2004; Lorraine-Colwill 2002).

The results from this study indicated that target-site mechanisms including point-mutations (Pro_{106} to Ser and Thr_{102} to Ile) or amplification of the *EPSPS* gene did not contribute to the mechanism of glyphosate resistance in a common ragweed biotype from Nebraska. These results are in consensus with shikimate accumulation, suggesting that the EPSPS enzyme in the GR biotype was inhibited by glyphosate, though the level of sensitivity was reduced compared to the GS biotype. However, an increasing level of shikimate accumulation at higher glyphosate concentrations in the GR plants suggested the possibility of non-target site mechanisms (Figure 2.1). Absorption and translocation

experiments revealed that total glyphosate absorption was the same in both common ragweed biotypes, but a more rapid rate of absorption was observed in the GS biotype compared to the GR biotype (Figure 2.3A). In contrast, overall translocation was slightly higher in the GR biotype (Figure 2.3B); however, the time required to complete 90% of the translocation was 2.6 times greater in the GR compared to the GS biotype (Table 2.3). Earlier studies have categorized common ragweed as a species with little glyphosate uptake (Sammons and Gaines 2014), and it was speculated that the plasma membrane transporters mediate the glyphosate exclusion from the plant cells in GR common ragweed (Ge et al. 2013). The slow rate of translocation in the GR biotype likely prevents the accumulation of glyphosate concentration in the cells or the chloroplast required to inhibit the *EPSPS* and completely block the shikimate pathway enough to cause plant mortality (Sammons and Gaines 2014).

The molecular mechanism of the slow absorption and translocation is not clear; however, the possibilities may include the presence of barriers interfering with glyphosate loading into the phloem or within cell movement and subcellular distribution. Several processes of non-target site mechanisms of glyphosate resistance have been reported. For example, the role of adenosine triphosphate (ATP)-binding cassette (ABC) transporters in the sequestration of glyphosate into vacuoles (Ge et al. 2010) or the upregulation of several *ABC transporter* genes has been reported in GR horseweed (Nol et al. 2012; Peng et al. 2010). Additionally, a recent study in GR hairy fleabane reported that glyphosate was not able to reach the target enzyme despite its presence in the cells due to impaired subcellular distribution that resulted in glyphosate inaction (Kleinman and Rubin 2016).

In conclusion, a non-target site-based resistance mechanism with restricted accumulation of glyphosate at the target enzyme due to slow rates of absorption and translocation has evolved in a GR common ragweed biotype from Nebraska. However, further research is needed to examine the differences in subcellular distribution of glyphosate and tonoplast membrane transporters between GR and GS common ragweed biotypes.

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Table 2.1. Primers used for sequencing conserved region of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) and for real-time quantitative PCR (qPCR) in glyphosate-resistant and -susceptible common ragweed biotypes from Nebraska.

Gene	Primer sequence	Amplicon size (bp)	Tm (C)	Reference
EPSPS (Thr_{102}, Pro_{106})	F- 5ʿ ATGTTGGACGCTCTCAGAACT -3ʿ R- 5ʻ TGAATTTCCTCCAGCAACGGC -3ʿ	195	56	Gaines et al. 2010
EPSPS (qPCR)	F- 5ʿ ATGTTGGACGCTCTCAGAACTCTTGGT -3ʿ R- 5ʻ TGAATTTCCTCCAGCAACGGCAA -3ʿ	195	59	Gaines et al. 2010
EPSPS (qPCR)	F- 5ʿ AGGGTTGTGGTGGTCTGTTTCC -3ʿ R- 5ʿ ATTTTCCTCCAGCAACGGCAAC -3ʿ	123	59	Ganie et al. (Unpublished)
β-*tubulin*	F- 5ʿ ATGTGGGATGCCAAGAACATGATGTG -3ʿ R- 5ʻ TCCACTCCACAAAGTAGGAAGAGTTCT -3ʿ	157	59	Godar et al. 2015

Table 2.2. Nucleotide bases and predicted amino acid sequence of the conserved region of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene covering Thr102 and Pro106 from glyphosate-resistant and -susceptible common ragweed biotypes from Nebraska.

Amino acid number Amino acid name	97 Leu	98 Gly	99 Asp	100 Ala	101 Gly	102 Thr	103 Ala	104 Met	105 Arg	106 Pro	107 Leu	108 Thr	109 Ala	110 Ala	111 Val
Consensus sequence [a]	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
Reference 1 (Palmer amaranth) Accession number FJ861243.1	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
Reference 2 (Spiny amaranth) Accession number KF569211.1	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR1	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR2	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR3	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR4	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR5	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR6	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR7	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GR8	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GS1	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GS2	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GS3	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GS4	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GS5	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GS6	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT
GS7	CTT	GGT	AAT	GCA	GGA	**ACA**	GCG	ATG	CGC	**CCA**	TTG	ACA	GCT	GCG	GTT

[a] Abbreviations: GR, glyphosate-resistant; GS, glyphosate-susceptible.

Table 2.3. Regression parameters for the absorption and translocation of 14C-glyphosate in glyphosate-susceptible and -resistant common ragweed biotypes from Nebraska. [a,b]

Movement of ^{14}C-glyphosate	Common ragweed biotype	Regression parameters[c]	
		A_{max}	t_{90}
Absorption into treated leaf	Susceptible	84 (1.0)	22 (2.0)
	Resistant	82 (1.5)	31 (5.5)
P-value		0.07	0.016
Total translocation into plant	Susceptible	73 (2.4)	26 (8.0)
	Resistant	84 (3.0)	69 (13.0)
P-value		0.015	0.011
Translocation to tissues above the treated leaf	Susceptible	14 (0.6)	27 (12.0)
	Resistant	13 (1.0)	64 (7.0)
P-value		0.102	0.033
Translocation to above ground tissues below the treated leaf	Susceptible	6 (0.4)	3 (1.0)
	Resistant	5 (0.4)	6 (1.0)
P-value		0.05	0.035
Translocation to roots	Susceptible	15 (1.4)	9 (5.0)
	Resistant	17 (2)	12 (6.0)
P-value		0.131	0.407
Translocation out of the treated leaf	Susceptible	27 (2.3)[d]	28 (7.1)
	Resistant	23 (1.6)[d]	44 (2.9)
P-value		0.094	0.008

[a] Parameter estimates for the rectangular hyperbolic model fit to the absorption and translocation data $y = \frac{(A_{max} \times t)}{(0.11 \times t_{90} + t)}$ where y is the percentage of the applied ^{14}C-glyphosate absorbed or translocated in the plant, A_{max} is the asymptote or maximum absorption or translocation expressed as the percent applied, t is the time (h) after herbicide application and t_{90} is the time required for 90% of the maximum absorption or translocation to occur.

[b] The predicted parameters of the glyphosate-resistant and -susceptible biotype were compared using the t-test and the P-values are presented.

[c] Values in parentheses are standard errors.

[d] Lower asymptote since ^{14}C-activity decreases in the treated leaf as translocation proceeds.

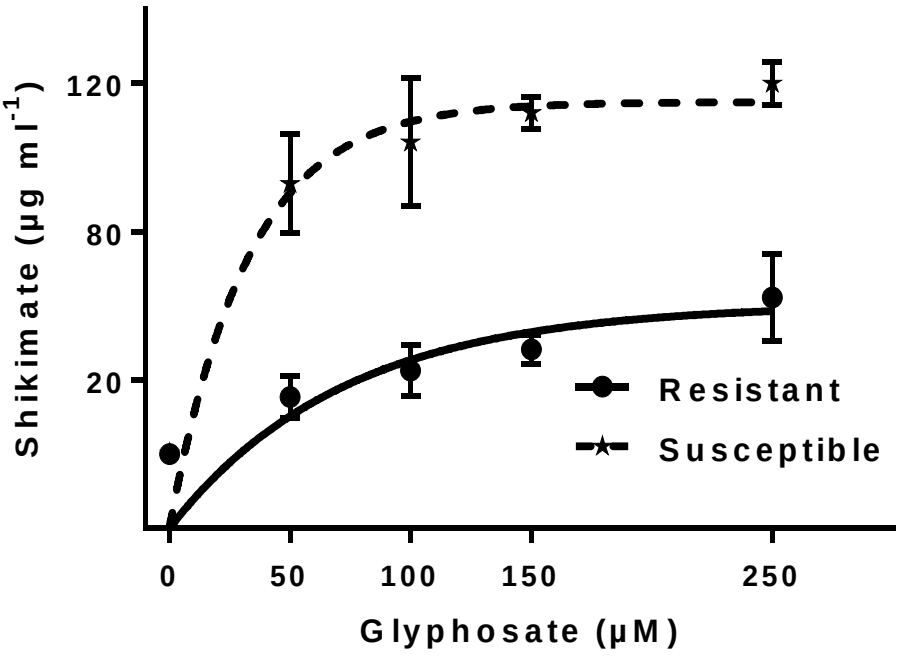


Figure 2.1. Accumulation of shikimate in leaf discs of the glyphosate-resistant and -susceptible common ragweed biotypes at 24 h after treatment (HAT) with increasing glyphosate concentrations. Each data point represents the mean amount of shikimate accumulation pooled from three experiments each with three replicates at each glyphosate concentration ± standard error (SE).

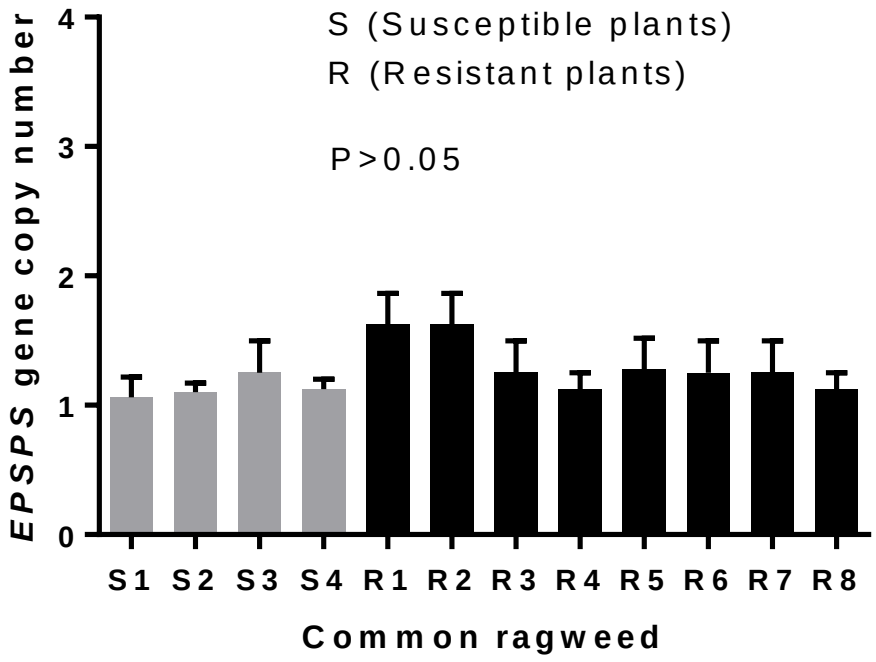


Figure 2.2. 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene copy number in glyphosate-susceptible (GS) and -resistant (GR) biotypes from Nebraska. EPSPS gene copy number was measured relative to a calibrator sample (S1). Error bars represent ± SE from the mean (n = 3 technical replicates). The real-time quantitative PCR (qPCR) data were normalized using β-tubulin as a reference gene.

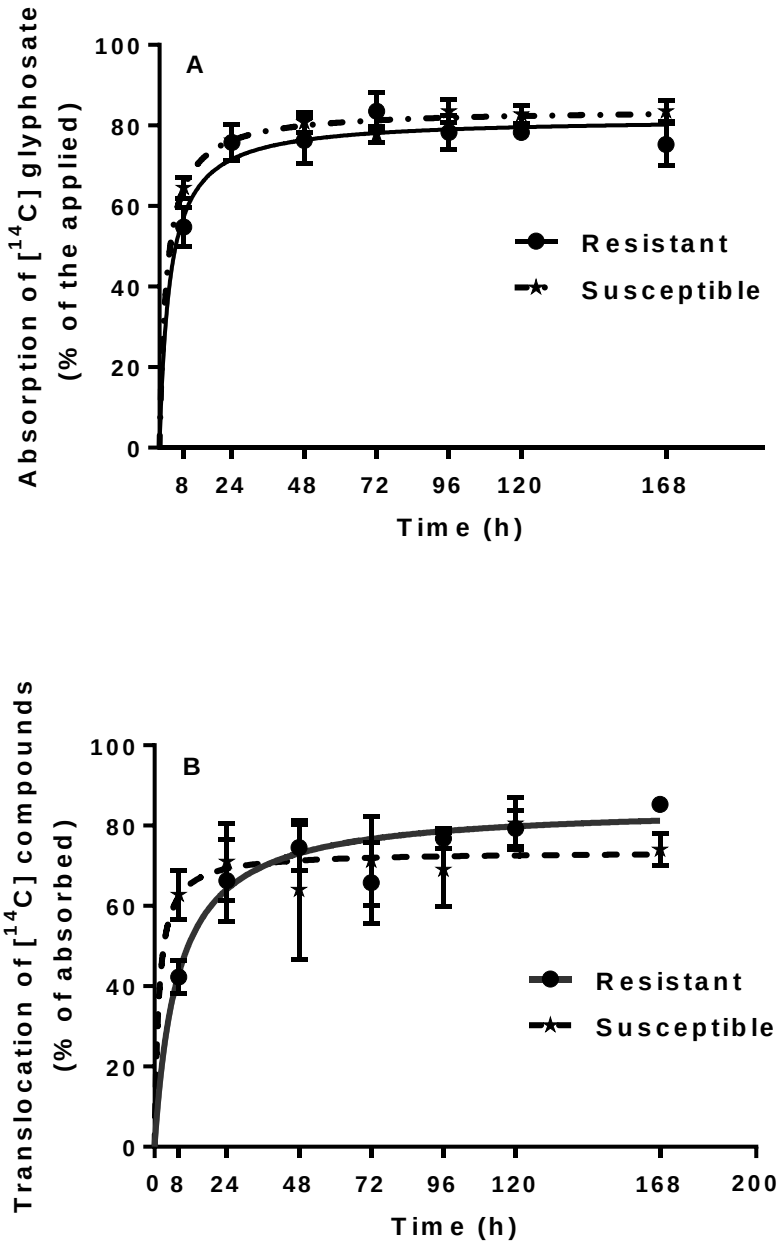
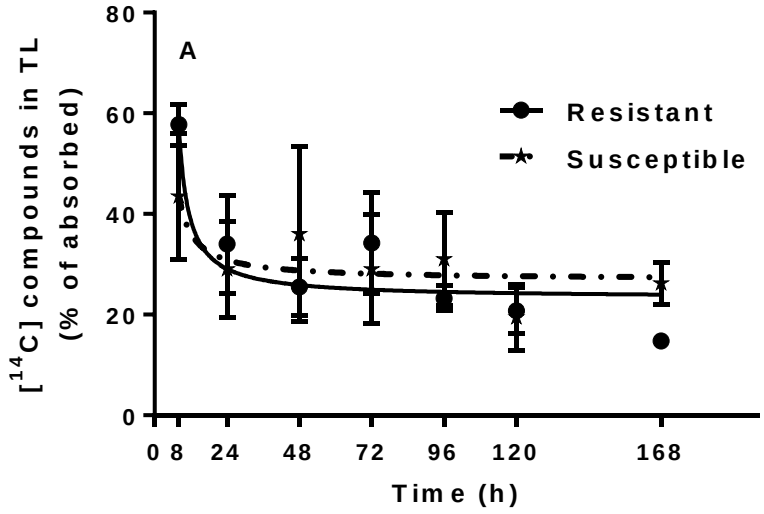
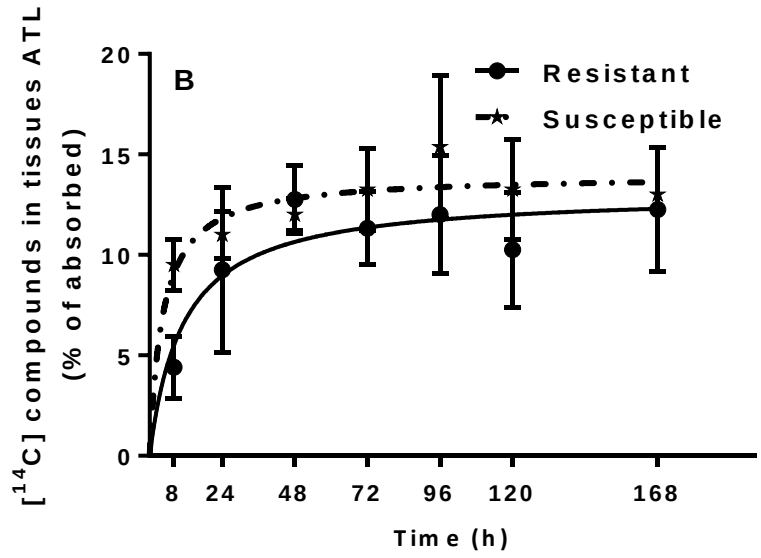


Figure 2.3. A pattern of 14C-glyphosate (A) absorption and (B) translocation in glyphosate-resistant and -susceptible common ragweed biotypes from Nebraska. Each data point represents the means based on two experiments each with four replicates. Vertical bars are the standard error of mean.


A
Resistant
Susceptible
[14C] compounds in TL
(% of absorbed)
80
60
40
20
0
0 8 24 48 72 96 120 168
Time (h)


B
Resistant
Susceptible
[14C] compounds in tissues ATL
(% of absorbed)
20
15
10
5
0
8 24 48 72 96 120 168
Time (h)

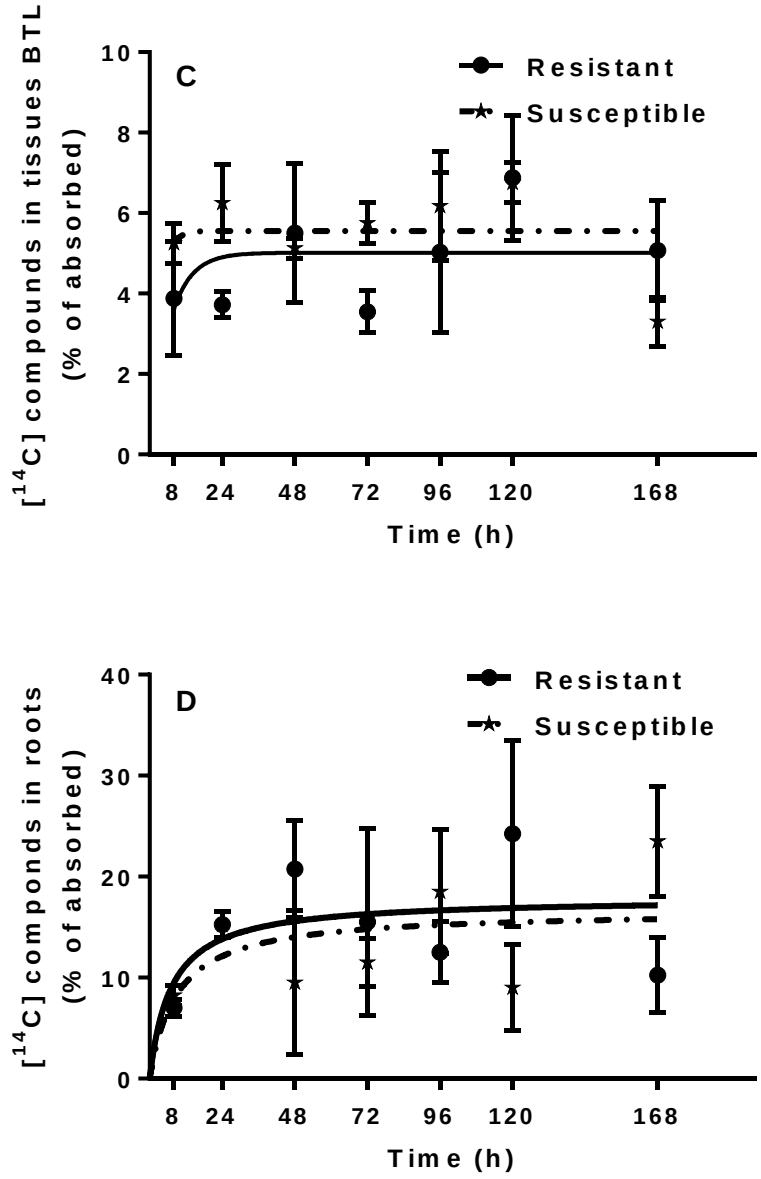


Figure 2.4. Percentage of ^{14}C-glyphosate translocated to plant sections including (A) treated leaf (TL), (B) tissues above treated leaf (ATL), (C) above-ground tissues below treated leaf (BTL), and (D) roots at different harvest time points [8, 24, 48, 72, 96, 120, and 168 h after treatment (HAT)] after the application of ^{14}C-glyphosate to glyphosate-resistant and -susceptible common ragweed biotypes from Nebraska. Each data point represents the mean based on two experiments each with four replicates. Vertical bars are the standard error of mean.

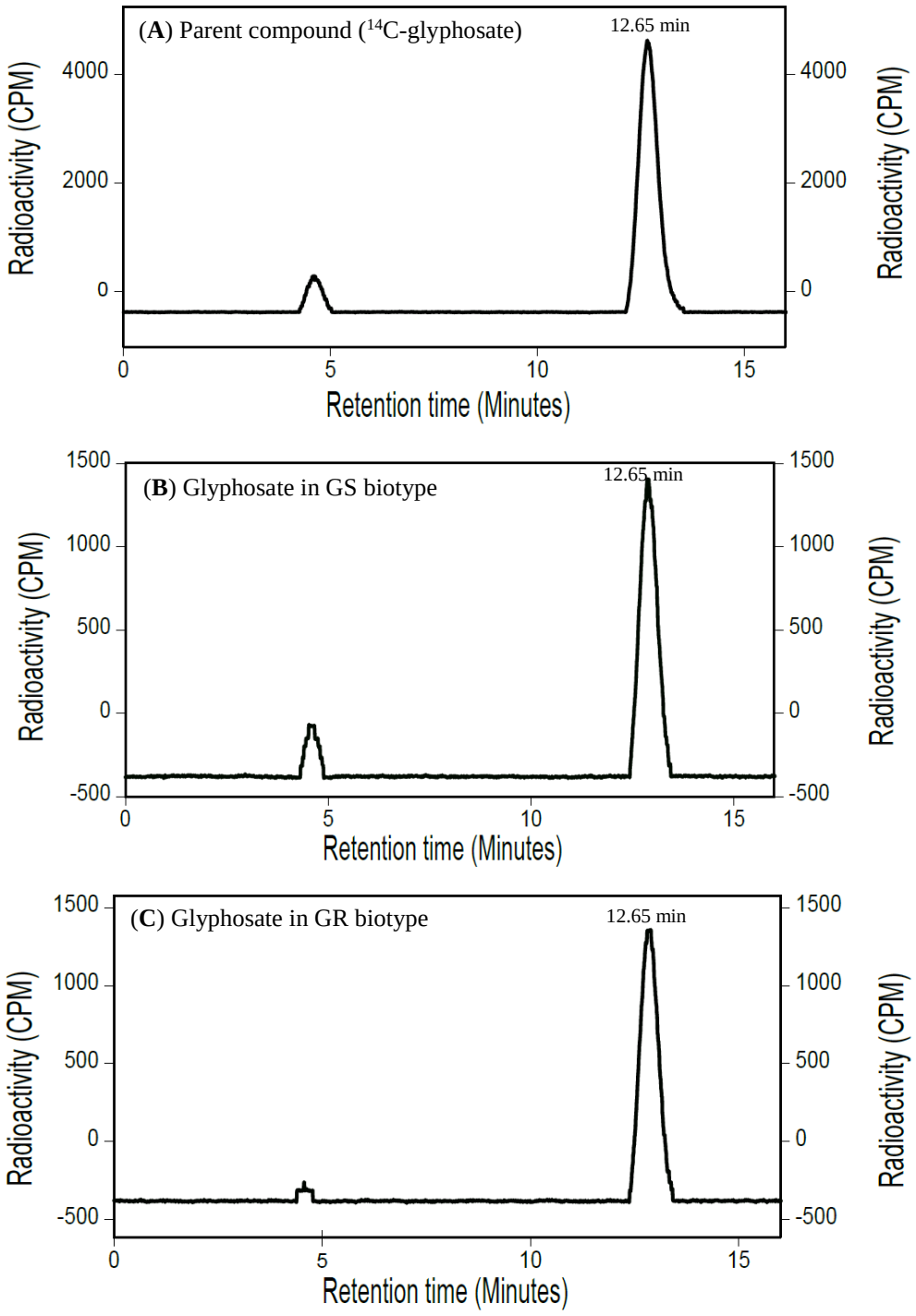


Figure 2.5. Reverse-phase HPLC chromatograms of glyphosate in glyphosate-susceptible (GS) and –resistant (GR) common ragweed at 96 h after treatment. The same peak retention time of 12.65 min for parent-compound ^{14}C-glyphosate (A), ^{14}C-glyphosate in extract from GS (B), and GR (C) biotypes indicates that glyphosate metabolism is not the mechanism in this biotype.

CHAPTER 3: EFFICACY, ABSORPTION, AND TRANSLOCATION OF 2,4-D OR GLYPHOSATE IN COMMON AND GIANT RAGWEED AT VARYING GROWTH TEMPERATURES

Abstract

Glyphosate and 2,4-D are very effective for the control of common and giant ragweed before planting corn and soybean in the Midwest; however, environmental factors including temperature may influence the efficacy of these herbicides. The objectives of this study were to evaluate the efficacy of 2,4-D or glyphosate for control of common and giant ragweed under different growth temperatures and to determine the underlying physiological mechanisms (absorption and translocation). An additional objective was included to determine the influence of growth temperatures on the level of glyphosate resistance in glyphosate-resistant common and giant ragweed biotypes. Glyphosate-susceptible and –resistant common and giant ragweed biotypes were used for 2,4-D or glyphosate dose-response studies at two growth temperatures (day/night, ^{0}C): low (LT) 20/11 and high (HT) 29/17. The results suggested an improved efficacy of 2,4-D or glyphosate at HT compared to LT for common and giant ragweed control, regardless of susceptibility or resistance to glyphosate. The level of glyphosate resistance decreased in both common and giant ragweed at HT. Absorption and translocation experiments indicated more translocation of 2,4-D in common and giant ragweed at HT compared to LT. Similarly, higher translocation in common ragweed, and increased absorption and translocation in giant ragweed resulted in greater efficacy of glyphosate at HT compared to LT. In conclusion, the efficacy of 2,4-D or glyphosate for common and giant ragweed

control can be improved if applied at warm temperature (29/17 ^{0}C d/n) due to increases in absorption and/or translocation of these herbicides compared to cooler temperatures (20/11 ^{0}C d/n).

Introduction

Common ragweed (*Ambrosia artemisiifolia* L.) and giant ragweed (*Ambrosia trifida* L.) are important broadleaf annual weeds of the Asteracea family native to the United States (Abul-Fatih and Bazzaz 1979; Bassett and Crompton 1975). Common and giant ragweed are widely distributed in diverse agroecosystems including waysides, low fertility areas, field edges and agronomic fields (Johnson et al. 2006; Jordan et al. 2007). Early spring emergence is a typical characteristic of common and giant ragweed in Nebraska (Kaur et al. 2016); therefore, preplant control with herbicides has been reported as the most effective method for the management of early season ragweed infestation (Ganie et al. 2016; Johnson et al. 2006; Jordan et al. 2007; Kaur et al. 2014). Nevertheless, follow-up PRE and/or POST herbicides are required for an effective season-long control of ragweed species in corn and soybean (Ganie et al. 2016; Jhala et al. 2014; Johnson et al. 2006; Jordan et al. 2007). Glyphosate has been the most commonly used herbicide for preplant or POST control of ragweed species in glyphosate-resistant (GR) corn or soybean in the Midwest; however, the evolution of biotypes of ragweed species resistant to glyphosate and/or acetolactate synthase (ALS)-inhibitors have severely reduced the number of control options (Chandi et al. 2012; Heap 2016; Patzoldt et al. 2001; Patzoldt and Tranel 2002; Regnier et al. 2016).

Growth regulator herbicides such as 2,4-D are effective for controlling common and giant ragweed (Ganie et al. 2016; Johnson et al. 2006; Jordan et al. 2007; Loux

2008). Ganie et al. (2016) and Jhala et al. (2014) reported ≥ 87% control of glyphosate-resistant (GR) giant ragweed at 14 d after treatment (DAT) with preplant burndown application of 2,4-D amine. Similarly, 2,4-D choline plus glyphosate resulted in > 93% control of GR common ragweed in the greenhouse at 21 DAT (Ganie et al. 2015a). However, the continuing evolution of new herbicide-resistant weeds, particularly those resistant to multiple herbicide sites-of-action, are severely reducing the number of effective herbicide options (Tranel et al. 2011). In addition, considering the stagnation in the discovery of herbicides with the new site-of-action for over three decades now (Duke 2012), it has become important to attain the best possible use of available herbicide active ingredients by making applications at the optimal weed growth stage and appropriate environmental conditions (Godar et al. 2015). Herbicide efficacy is affected by plant characteristics including plant type and/or growth stage (Ganie et al. 2015b; Chahal et al. 2015), along with environmental factors such as light intensity, temperature, water stress, relative humidity, nutrient status, and atmospheric pollution (Anderson et al. 1993; Cole 1983; Gerber at al. 1983; Godar et al. 2015; Hull 1970; Johnson and Young 2002; Price 1983). Previous studies have reported that growth temperature before, during, or after herbicide application has a major influence on herbicide efficacy: for example, glyphosate resulted in greater control of johnsongrass [*Sorghum halepense* (L.) Pers.] at 35 C compared to 24 C (McWhorter 1980). Similarly, the efficacy of glyphosate on bermudagrass [*Cynodon dactylon* (L.) Pers.] improved at 32 C compared to 22 C at 40% relative humidity (Jordan 1977). In contrast, mesotrione showed higher efficacy for the control of common waterhemp (*Amaranthus rudis* Sauer) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.] at 18 C compared to 32 C (Johnson and Young 2002). Godar et

al. (2015) reported that Palmer amaranth (*Amaranthus palmeri* S. Wats.) was more sensitive to mesotrione at low (25/15 C d/n) compared to high temperatures (40/30 C d/n). Increased absorption or translocation at higher temperatures usually results in improved herbicide efficacy (Pline et al. 1999); whereas, relatively improved efficacy at lower temperatures might be due to a slower metabolism (Godar et al. 2015).

The level of glyphosate resistance may vary with the growth temperature at which the weed species is grown. Hairy fleabane (*Conyza bonariensis*) showed 2- to 10-fold more resistance to glyphosate at high temperature regimes (28/22 or 34/28 C) compared to low temperature regimes (16/10 or 22/16 C) (Kleinman et al. 2011). Vila-Aiub et al. (2013) reported that the level of glyphosate resistance in johnsongrass [*Sorghum halepense* (L.) Pers.] and rigid ryegrass (*Lolium rigidum* Gaudin) varied with the growth temperature, and these species were relatively more susceptible (50 to 70%) to the labeled rate of glyphosate at 19 and 8 C compared to < 50 or 40% control at 30 and 19 C, respectively. Similarly, Nguyen et al. (2015) reported an increase in the level of glyphosate resistance in barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] at 30 C compared to 20 C, due to a 2-fold increase in glyphosate uptake at 20 C in both GR and glyphosate-susceptible (GS) biotypes.

Preplant burndown herbicides such as glyphosate and/or 2,4-D are applied early in the spring typically from March 15 to May 10 in the Midwest for control of winter annual weeds such as henbit (*Lamium amplexicaule* L.), field pennycress (*Thlaspi arvense* L.), horseweed [*Conyza canadensis* (L.) Cronq.], etc., as well as some early spring weeds such as common and giant ragweed (Jhala 2016). The daily temperature during early spring is highly variable (Figure 3.1), and may have an effect on weed

growth and development (Leon et al. 2004, Schwabe 1957, Milthrope 1956) and more likely on the efficacy of preplant burndown herbicides (Godar et al. 2015, Hammerton 1967). Scientific literature is not available on the effect of varying temperatures on the efficacy of 2,4-D or glyphosate for control of common and giant ragweed. Therefore, we hypothesized that lower temperatures will decrease the efficacy of preplant herbicides, including 2,4-D or glyphosate on early emerging weed species including common and giant ragweed. The objectives of this study were to 1) evaluate the efficacy, absorption, and translocation of 2,4-D or glyphosate on common and giant ragweed at varying growth temperatures, and 2) determine the effect of varying growth temperatures on the level of glyphosate resistance in common and giant ragweed.

Materials and Methods

Plant Material and Growth Conditions. The GR common ragweed biotype was collected from a grower's field (40.44°N, 96.62°W) in Gage County, NE. The GR giant ragweed biotype was collected from a grower's field (41.25°N, 97.13°W) in Butler County, NE. The level of resistance in the common and giant ragweed biotypes were 19- and 14-fold, respectively, compared with the known GS biotypes (Ganie et al. 2015; Rana et al. 2013). GS biotypes of common and giant ragweed were collected from the University of Nebraska-Lincoln, South Central Agricultural Laboratory, Clay Center, NE (40.52°N, 98.05°W) and were used in this study for comparison. The seeds were cleaned and stored at 4 C until used in this study. Giant ragweed has seed dormancy and relatively low germination rates (Page and Nurse 2015). To break the seed dormancy, seeds were packed in mesh bags and stratified by placing them in between layers of a mixture of potting mix and soil (3:1) in plastic boxes (58 x 42 x 15 cm), which were then

kept in a freezer for 3.5 months. GR and GS common and giant ragweed seeds were germinated in plastic trays (25 × 15 × 5-cm) filled with commercial potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, Quebec, Canada) and uniform-sized individual seedlings were transplanted at the two-leaf stage into square plastic pots (6 × 6 × 6.5-cm) containing a 3:1 mixture of potting mix to soil. The plants were supplied with adequate water every day and fertilized once a wk after transplanting. The growth conditions in the greenhouse were maintained at 26/21 C day/night temperature, 65 ± 5% relative humidity, and a 15-h photoperiod supplemented with sodium vapor lamps providing 120 μmol $m^{-2}s^{-1}$ photon flux. After 4 to 5 d of transplanting, healthy uniform-sized plants (5 to 6 cm tall) were transferred to growth chambers that were maintained at two day/night (d/n) temperature regimes: low temperature (LT; 20/11 C) and high temperature (HT; 29/17 C). The transition of temperatures between day and night or vice versa were programmed to start progressively over a 2-h period to reach the set value without causing abrupt temperature shock to the plants. Plants were maintained at a 15/9 h day/night length and the light sources in the growth chambers were incandescent and fluorescent bulbs delivering 550 μmol $m^{-2}s^{-1}$ photon flux at the plant canopy level. All of the growth chambers were maintained at 70 ± 5% relative humidity throughout the experiment and the plants were watered regularly.

Dose-Response Experiments. GR and GS biotypes of both common ragweed and giant ragweed plants (grown under conditions described earlier) were treated with different rates of glyphosate (Roundup PowerMax, Monsanto Company, 800 North, Lindberg Ave., St. Louis, MO) when the plants were 10 to 12 cm tall (8 to 10 leaf stage). GR biotypes of common ragweed or giant ragweed were used in dose-response study with

2,4-D amine (Winfield Solutions, LLC, St Paul, MN 55164). Glyphosate or 2,4-D was applied at rates of 0, 0.06×, 0.12×, 0.25×, 0.5×, 1×, 2×, and 4×, where × is 560 g ae ha^{-1} for 2,4-D or 1,260 g ae ha^{-1} for glyphosate. An additional 8× rate of glyphosate was used for the GR biotypes. The herbicide treatments were prepared in distilled water and nonionic surfactant (Induce, Helena Chemical Co., Collierville, TN) was added to both 2,4-D, and glyphosate at 0.25% v v^{-1}. Ammonium sulfate (DSM Chemicals North America Inc., Augusta, GA) was added to glyphosate treatments at 1% wt v^{-1}. The herbicide treatments were applied with an automated bench-type sprayer (Research Track Sprayer, De Vries Manufacturing, RR 1 Box 184, Hollandale, MN, USA) equipped with a flat-fan nozzle tip (80015LP TeeJet tip, Spraying Systems Co., Wheaton, IL, USA) delivering 187 L ha^{-1} at 207 kPa in a single pass at 4.8 km h^{-1}. The temperature, relative humidity, and light intensity at the time of herbicide application was 26 C, 65%, and 15 μmol m^{-2} s^{-1}, respectively. Plants were returned to their respective growth chambers within 30 min after treatment. The experiments were arranged in a split-plot design with two temperature regimes (LT and HT) as main plot treatments and herbicide rates as sub-plot treatments. The treatments were replicated four times and the experiment was repeated twice with the same procedure except that the growth chambers were switched.

Visual control assessments were recorded at 21 DAT using a 0 to 100% scale, with 0 equivalent to no control and 100% equivalent to complete control or mortality of the glyphosate- or 2,4-D-treated common ragweed and giant ragweed plants. Percent control estimates for treated plants were assessed based on a comparison to non-treated control plants with respect to symptoms such as twisting/epinasty (2,4-D), chlorosis, necrosis, stand loss, and stunting (glyphosate). Aboveground biomass of each plant was

cut close to the base at 21 DAT, oven dried at 65 C for three days, and weighed (g). The biomass data were converted into percent biomass reduction compared to the nontreated control (Ganie et al. 2015b; Wortman 2014) as:

$$\text{Percent biomass reduction} = [(\bar{C} - B)/\bar{C}]^{*}\,100 \qquad [1]$$

where $\bar{C}$ is the mean biomass of the six nontreated control replicates and B is the biomass of an individual treated experimental unit.

The dose-response experiments were arranged in a factorial combination with two levels of growth temperature (LT, HT) and eight levels of the herbicide treatments. Data were subjected to ANOVA in SAS to test the treatment-by-experiment interactions. Control estimates and biomass reduction data were regressed over herbicide treatments using a four-parameter log-logistic model in the *drc* package (drc 1.2, Christian Ritz and Jens Strebig, R2.5, Kurt Hornik, online) of R software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) (Ritz and Streibig 2005):

$$Y = \mathrm{L} + \{\mathrm{U} - \mathrm{L}/1 + \exp[\mathrm{S}\,(\log\mathrm{X} - \log\mathrm{E})]\} \qquad [2]$$

where Y is the response variable (percent control estimates or percent reduction in biomass), L is the lower limit, U is the upper limit, S is the slope of the curve, E is the dose resulting in 50% or 90% control (known as ED_{50} or ED_{90}), and X is the herbicide rate. This model was used to determine ED_{50} or ED_{90} (effective doses required for 50 or 90% control) and GR_{50} or GR_{90} (effective doses required for 50 or 90% biomass reduction) from the visual injury and biomass reduction data, respectively. The glyphosate-resistance level at varying growth temperatures was determined by dividing the ED_{90} and GR_{90} values with the recommended field rate of 1,260 g ae ha^{-1}.

Additionally, the ratio of the effective doses (ED_{50}, ED_{90}, GR_{50} and GR_{90}) of the glyphosate-resistant to the susceptible biotype (R/S ratio) are presented (Tables 3.1 and 3.2), but due to the greater sensitivity of the susceptible biotypes at HT, it may not be a stable measure of resistance level across the temperature regimes.

Absorption and Translocation Experiments. Uniform-sized common and giant ragweed seedlings grown in the greenhouse (as described earlier) were shifted to growth chambers maintained at LT or HT and allowed to acclimatize for 6 to 10 d. Eight to 10 cm tall plants were treated with ten 1-µL droplets of ^{14}C-labelled glyphosate (3.3 kBq with specific activity of 1.85 MBq $mmol^{-1}$) [PerkinElmer Inc. 549 Albany Street, Boston, MA] or 2,4-D (3.3 kBq with specific activity of 5.5 MBq $mmol^{-1}$) [Dow AgroScience 9330 Zionsville Road, Building 306-D2, Indianapolis, IN] on the upper surface of the fully expanded fourth youngest leaf. Commercial glyphosate or 2,4-D was added to the respective radioactive solutions to obtain the recommended 1× concentration equivalent to 1,260 g ae ha^{-1} of glyphosate or 560 g ae ha^{-1} of 2,4-D. The plants were returned to the growth chambers within 30 min of treatment. Subsequently, plants were dissected at 24, 48, 72, and 96 h after treatment (HAT) into treated leaf (TL), tissues above treated leaf (ATL), and tissues below treated leaf (BTL). Treated leaves were rinsed twice in a 20 ml scintillation vial containing 5 ml wash solution (1:1 v/v mixture of methanol and deionized water and 0.45% tween-20) for 1 min to remove the unabsorbed herbicide from the surface of the treated leaf. The leaf rinse was mixed with 15 ml of scintillation cocktail [Ecolite-(R), MP Biomedicals, LLC. Santa Ana, CA, USA], and the radioactivity was determined by using liquid scintillation spectrometry (LSS) (Tricarb 2100 TR Liquid Scintillation Analyzer; Packard Instrument Co., Meriden, CT). Plant sections were dried

at 55 C for 72 h and combusted in a biological oxidizer (OX-501, RJ Harvey Instrument, NY) for three min to recover ^{14}C labelled glyphosate or 2,4-D in a proprietary ^{14}C-trapping scintillation cocktail and radio-assayed using LSS. Herbicide absorption and translocation was calculated as (Godar et al. 2015):

$$\%\ Absorption = (\frac{Total\ radioactivity\ applied - Radioactvity\ recovered\ in\ wash\ solution}{Total\ radioactivity\ applied} \times 100 \qquad [3]$$

$$\%Translocation = 100 - \%\ Radioactivity\ in\ treated\ leaf \qquad [4]$$

where $\%\ Radioactivity\ in\ treated\ leaf = \frac{Radioactivity\ recovered\ in\ treated\ leaf}{Radioactivity\ absorbed} \times 100$

The experiments were arranged in a split-plot design with two growth temperatures (LT and HT) as main plot treatments and harvest time (HAT; hours after treatment) as sub-plot treatment. Within each temperature regime the experimental units were arranged in a randomized complete block design by blocking to overcome variability due to plant size with four replications and the experiment was repeated twice following the same procedure except that the growth chambers were switched. Data from absorption and translocation experiments were subjected to ANOVA in SAS version 9.3 (SAS Institute Inc, Cary, NC) using the PROC GLIMMIX procedure to test the treatment-by-experiment interaction. The absorption or translocation data was regressed over the harvest time using best fit linear model in GraphPad Prism 6 (GraphPad Software, Inc., Avenida de la Playa, La Jolla, CA 92037):

$$y = a + bx \qquad [5]$$

where y is the percentage of the applied ^{14}C-glyphosate absorbed or translocated in the plant, a is the intercept or initial absorption or translocation expressed as the percent

applied or absorbed, b is the slope or rate of change of the absorption/translocation over time, and x is the time expressed as hours after treatment (HAT).

Results and Discussion

The treatment-by-experiment interactions in the dose-response or the absorption and translocation studies were not significant ($P > 0.05$); therefore data were combined over the experiments.

2,4-D Dose-Response. The sensitivity of common and giant ragweed to 2,4-D varied with growth temperature (Figure 3.2). Effective doses to achieve 50% and 90% control (ED_{50}, ED_{90}) of common ragweed were 187 and 3,805 g ae ha^{-1} at LT compared to 61 and 177 g ae ha^{-1} at HT, respectively (Table 3.1). In contrast, the ED_{50} and ED_{90} for giant ragweed were 71 and 792 g ae ha^{-1} at LT compared to 13 and 49 g ae ha^{-1} at HT, respectively (Table 3.1). The biomass reduction results revealed that GR_{90} was 2.8 and 2.9 times less in common and giant ragweed, respectively, at HT compared to LT (Table 3.2). These results suggest that the efficacy of 2,4-D was improved with increase in d/n growth temperature from 20/11 C to 30/20 C in both common and giant ragweed. Previously, Kelly (1949) reported that kidney beans (*Phaseolus vulgaris* L.) were more sensitive to 2,4-D at 25 C compared to 5 or 15 C, and the biologically effective herbicide rates required at high temperature were relatively lower compared to the rates required at the low temperatures. Similarly, the response of flax (*Linum usitatissimum* L.) to 2,4-D improved with the increase in d/n temperature from 18/18 C to 24/18 C or 29/18 C (Jordan et al. 1960). However, the mortality of buckhorn plantain (*Plantago lanceolata* L.) with 2,4-D was faster at the optimum temperature (18 to 24 C), possibly due to better growth compared to lower (10 to 15 C) or higher (24 to 32 C) temperature regimes.

Glyphosate Dose-Response. The efficacy of glyphosate on both GS and GR common or giant ragweed improved at HT compared to LT (Figure 3.3 and 3.4). In GS common ragweed ED_{50} and ED_{90} were 437 and 6, 963 g ae ha^{-1} at LT compared to 130 and 587 g ae ha^{-1} at HT, respectively (Table 3.1). GR_{50} and GR_{90} were 45 and 1, 249 g ae ha^{-1} at LT compared to 39 and 210 g ae ha^{-1} at HT, respectively (Table 3.2). Similarly, the effective doses of glyphosate for GR common ragweed control were reduced at HT compared to LT, suggesting that the level of glyphosate resistance decreased at HT (Tables 3.1 and 3.2). Resistance level and R/S ratio in GR common ragweed decreased from 94 and 17 at LT to 6.6 and 14.2 at HT, respectively (Table 3.1). Similarly, the resistance level and R/S ratio determined from the biomass reduction data also suggested that the resistance to glyphosate was decreased with increasing growth temperature (Table 3.2).

Lower rates of glyphosate were required for both GS and GR giant ragweed biotypes for a similar level of control compared to common ragweed irrespective of the temperature (Tables 3.1 and 3.2). ED_{50} and ED_{90} in GS giant ragweed were 119 and 468 g ae ha^{-1} at LT compared to 62 and 244 g ae ha^{-1} at HT, respectively (Table 3.1). Similarly, GR_{50} and GR_{90} were 59 and 956 g ae ha^{-1} at LT compared to 49 and 154 g ae ha^{-1} at HT, respectively (Table 3.2). The effective doses required for control of GR giant ragweed were reduced at HT and the resistance level at LT was 52 compared to 4.6 at HT (Tables 3.1 and 3.2). Biomass reduction in GR giant ragweed showed consensus with the control estimates in response to glyphosate at both growth temperatures. GR_{50} and GR_{90} values for GR giant ragweed were 349 and 5,879 g ae ha^{-1} at LT compared to 218 and 2,293 g ae ha^{-1} at HT, respectively (Table 3.2). Masiunas and Weller (1988) reported greater phytotoxicity and reduced fresh weight accumulation following glyphosate

application at high d/n temperature regime (23/13 C) compared to a low temperature regime (13/4 C) in potato (*Solanum tuberosum* L.). Similarly, Jordan (1977) and Reddy (2000) reported an improvement in glyphosate efficacy on bermudagrass [*Cynodon dactylon* (L.) Pers.] and redvine [*Brunnichia ovata* (Walt.) Shinners], respectively, at high temperature compared to low temperatures.

2,4-D Absorption and Translocation. Absorption and translocation pattern of [^{14}C] 2,4-D varied with growth temperature in both common ragweed and giant ragweed (Figure 3.5). For example, mean absorption of [^{14}C] 2,4-D in common ragweed increased from 17 to 40% and 20 to 35% at LT and HT, respectively in a period of 24 to 96 HAT (Figure 3.5A). In contrast, [^{14}C] 2,4-D absorption increased from 30 to 58% at LT and 20 to 62% at HT in giant ragweed in a time period of 24 to 96 HAT (Figure 3.5C). However, translocation of [^{14}C] 2,4-D was greater at HT compared to LT in both common and giant ragweed, likely contributing to improved efficacy at HT. Mean translocation in common ragweed reached 54% of the absorbed [^{14}C] 2,4-D at HT compared to 35% at LT in 96 HAT (Figure 3.5B). Similarly, in giant ragweed 45% of the absorbed [^{14}C] 2,4-D was translocated at HT compared to 27% at LT at 96 HAT (Figure 3.5D). Increased translocation to both tissues above and below the treated leaf occurred at HT compared to LT (data not shown). Schultz and Burnside (1980) reported comparable translocation of 2,4-D varying from 35 to 39% at 25 and 30 C in hemp dogbane (*Apocynum cannabinum* L.). However, increased absorption and translocation of 2,4-D was reported in kidney beans with an increase in temperature from 20 to 30 C (Pallas 1960).

Glyphosate Absorption and Translocation. Mean absorption of [^{14}C] glyphosate (as % applied) increased from 49 to 64% at LT compared to 41 to 55% at HT in GS common

ragweed in a period of 24 to 96 HAT (Figure 3.6A). However, in 96 HAT translocation of absorbed [^{14}C] glyphosate reached 54% at HT compared to 35% at LT (Figure 3.6B). In contrast, the absorption of [^{14}C] glyphosate in GS giant ragweed varied from 18 to 54% at HT compared to 11 to 40% at LT (Figure 3.7A). At 96 HAT 69% of the absorbed [^{14}C] glyphosate was translocated at HT compared to 50% at LT in GS giant ragweed (Figure 3.7B). These results suggested that the absorbed [^{14}C] glyphosate was translocated at a higher rate in common ragweed at HT compared to LT. However, in giant ragweed, a higher rate of both absorption and translocation was observed at HT compared to LT. Schultz and Burnside (1980) also reported that glyphosate translocation increased from 18% at 25 C to 39% at 30 C in hemp dogbane. Similarly, Reddy (2000) reported an increase in absorption and translocation of glyphosate in redvine at a d/n temperature of 35/30 C compared to 25/20 or 15/10 C.

The glyphosate dose-response results suggested a decrease in the glyphosate resistance level at HT compared to LT in both GR common or giant ragweed. The mean absorption of [^{14}C] glyphosate in GR common ragweed increased from 47 to 60% at LT compared to 39 to 60% at HT in a period of 24 to 96 HAT (Figure 3.6C). Conversely, higher [^{14}C] glyphosate translocation was observed in common ragweed at HT varying from 25 to 41% compared to 17 to 33% at LT (Figure 3.6D). Likewise, the mean absorption of [^{14}C] glyphosate in GR giant ragweed increased from 15 to 34% at HT and 15 to 23% at LT (Figure 3.7C). However, translocation of the absorbed [^{14}C] glyphosate increased from 22 to 41% at HT compared to 12 to 44% at LT in GR giant ragweed (Figure 3.7D). Increased absorption and/or translocation of [^{14}C] glyphosate reduced the level of glyphosate resistance at HT though the GR biotypes of common and giant

ragweed did not become susceptible as the effective glyphosate rates required for 90% control were still higher compared to the labelled rate (Tables 3.1 and 3.2). Pline et al. (1999) reported that resistance in transgenic GR soybeans decreased at 35 C due to an increase in the translocation of glyphosate to the meristematic regions.

Results indicate that increasing efficacy of 2,4-D or glyphosate for control of common or giant ragweed at HT compared to LT, and the level of glyphosate resistance was reduced in both GR common and giant ragweed at HT. In addition, the herbicide rates required for the same level of control for both ragweed species were lower at HT compared to LT (Tables 3.1 and 3.2). Muzik and Mauldin (1964) suggested that both absorption and translocation of the systemic herbicides might be enhanced at high temperatures due to the effect of temperature on herbicide penetration facilitated by physicochemical factors including increased rate of diffusion, reduced viscosity of the cuticle, and physiological factors comprising increases in photosynthesis, phloem translocation, and protoplasmic streaming and growth (Currier and Dybing 1959). It has also been reported that increased growth temperatures modify the characteristics of leaf cuticular wax (Hess and Falk 1990; Willingham and Graham 1988) and enhance the cuticle and plasma membrane fluidity, resulting in improved herbicide absorption and translocation (Johnson and Young 2002). Additionally, studies have suggested that the rate of photosynthesis increases at HT resulting in a higher production of photosynthates, faster loading of the systemic herbicides into phloem along with the photosynthates, and enhancement of the rate of herbicide translocation and distribution within the plant (Bromilow et al. 1993; Pline et al. 1999).

The absorption and translocation of 2,4-D in this study suggested that increased translocation possibly contributed to the higher efficacy in both common and giant ragweed at HT compared to LT. Several studies reported increase in 2,4-D efficacy with rise in the growth temperature in species including common duckweed (*Lemna minor* L.) (Blackman and Robertson-Cunninghame 1955), buckhorn plaintain (Marth and Davis 1945), flax (Jordan et al. 1960), and beans (Pallas 1960). However, the mechanism(s) for increased efficacy of 2,4-D at warmer temperatures has not been studied thoroughly; nonetheless, slower uptake and translocation combined with detoxification of 2,4-D were suspected as a possible mechanism for reduced efficacy at lower temperature in fiddleneck [*Amsinckia intermedia* (Fisch. & C. A. Mey.)] (Muzik and Mauldin 1964). Similarly, greater efficacy of glyphosate at HT compared to LT in this study can be attributed to increased translocation of this herbicide in common ragweed, and increased absorption as well as translocation in giant ragweed. Schultz and Burnside (1980) reported reduced tolerance in hemp dogbane at 30 C compared to 25 C due to an increase in glyphosate translocation at 30 C. Earlier studies have reported that increase in glyphosate absorption with rise in temperature resulted in greater phtotoxicity at warm temperature compared to lower temperature regimes in potato (Masiunas and Weller 1988). Furthermore, increase in both glyphosate absorption and translocation with rise in temperature has been reported in johnsongrass [*Sorghum halepense* (L.) Pers.] (McWhorter et al. 1980).

The sensitivity of common and giant ragweed to 2,4-D or glyphosate increased with a rise in temperature; therefore, temperature should be considered determining the proper time of application of preplant herbicides such as 2,4-D or glyphosate for control

of early emerged ragweed plants. Since daily temperatures fluctuate widely in the spring (Figure 3.1), applications of 2,4-D or glyphosate should be scheduled for warmer days to improve their efficacy. In addition, temperature forecasts for the days following herbicide application should be warmer for improved efficacy. This study was conducted under growth chamber conditions with precise temperature regimes with constant relative humidity; therefore, results of this study may vary under field conditions due to the complex interaction of diverse environmental factors including fluctuation in temperature, relative humidity, wind, light, etc.on herbicide efficacy. Stopps et al. (2013) reported that glyphosate efficacy on velvetleaf, pigweed, and common ragweed increased when treatments were applied between noon and 6 PM, which corresponds with the maximum air temperatures observed during the day. Future studies should be conducted to evaluate the simultaneous effect of temperature and other environmental factors such as light and relative humidity on herbicide efficacy, and molecular studies including changes in gene expression with varying environmental factors may reveal further details about the observed physiological mechanisms.

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Table 3.1. Estimates of regression parameters and 2,4-D or glyphosate doses required for 50% (ED_{50}) and 90% (ED_{90}) control of glyphosate-resistant and -susceptible common ragweed and giant ragweed at 21 d after treatment (DAT) in whole-plant dose response studies conducted at high and low temperature regimes in growth chambers.

Herbicide	Low temperature (d/n 20/11 C)[a]					High temperature (d/n 30/20 C)[a]				
	Regression parameters [b]			Effective herbicide doses		Regression parameters [b]			Effective herbicide doses	
	S	L	U	ED_{50} (±SE)	ED_{90} (±SE)	S	L	U	ED_{50} (±SE)	ED_{90} (±SE)
				g ae ha^{-1}					g ae ha^{-1}	
Common ragweed										
2,4-D	0.6 (0.2)	-2.0 (1.0)	103 (4)	187 (22)	3,805 (166)	1.9 (0.2)	1.2 (0.7)	101 (2)	61 (4)	177 (21)
Glyphosate										
GS biotype	0.8 (0.2)	0.7 (0.4)	102 (6)	437 (50)	6,963 (2,159)	1.5 (0.2)	1.5 (0.8)	101 (3)	130 (11)	587 (96)
GR biotype	0.7 (0.2)	-1.6 (1.0)	73 (13)	2,821 (343)	1,18,371 (35,427)	1.2 (0.3)	1.8 (1.0)	97 (10)	1,307 (140)	8,354 (2,145)
Resistance level [c]	-	-	-	-	94.0	-	-	-	-	6.6
R/S ratio	-	-	-	6.5	17.0	-	-	-	10.0	14.2
Giant Ragweed										
2,4-D	0.8 (0.2)	-3.3 (2.3)	104 (8)	71 (11)	792 (192)	1.6 (0.1)	-1.9(1.0)	99 (0.30)	13 (1)	49 (1.7)
Glyphosate										
GS biotype	1.8 (0.3)	0.3 (0.2)	100 (2.5)	119 (15)	468 (168)	1.7 (0.3)	0.0 (0.0)	99 (1.6)	62 (5)	244 (35)
GR biotype	0.8 (0.5)	4.6 (2.9)	77 (2.4)	1,429 (280)	66,207 (20,918)	1.9 (1.4)	3.5 (2.0)	86 (3.7)	1,164 (144)	5,751 (1,445)
Resistance level [c]	-	-	-	-	52	-	-	-	-	4.6
R/S ratio	-	-	-	12.0	141.5	-	-	-	18.8	23.6

[a] Abbreviations: d/n, day/night temperatures; ED_{50}, effective 2,4-D or glyphosate dose required for 50% control of common or giant ragweed; ED_{90}, effective 2,4-D or glyphosate dose required for 90% control of common or giant ragweed; SE, standard error.

[b] Regression parameters S (slope), L (lower limit) and U (upper limit) of the four-parameter log-logistic model ($Y = L + \{U - L/1 + \exp[S(\log X - \log E)]\}$) were determined by using the nonlinear least-square function of the statistical software R.

[c] The resistance level was determined compared to the field rate of glyphosate (i.e., 1,260 g ae ha^{-1}) because the glyphosate-susceptible (GS) biotypes became too sensitive at the high temperature regime, leading to instability in the resistance level determined on the basis of the R/S ratio.

Table 3.2. Estimates of regression parameters and 2,4-D or glyphosate doses required for 50% (ED_{50}) and 90% (ED_{90}) biomass reduction of glyphosate-resistant and -susceptible common ragweed and giant ragweed at 21 d after treatment (DAT) in greenhouse whole-plant dose response studies conducted at high and low temperature regimes.

Herbicide	Low temperature (d/n 20/11 C)[a]					High temperature (d/n 30/20 C)[a]				
	Regression parameters [b]			Effective herbicide doses		Regression parameters [b]			Effective herbicide doses	
	S	L	U	GR_{50} (±SE)	GR_{90} (±SE)	S	L	U	GR_{50} (±SE)	GR_{90} (±SE)
				g ae ha^{-1}					g ae ha^{-1}	
Common ragweed										
2,4-D	0.6 (0.2)	-0.9 (0.3)	81 (7)	20 (8)	365 (85)	0.8 (0.4)	-0.7 (0.1)	87 (4)	17 (7)	128 (59)
Glyphosate										
GS biotype	0.7 (0.3)	-0.5 (3.0)	87 (7)	45 (17)	1,249 (246)	1.3 (0.4)	-0.7 (0.5)	97 (2)	39 (9)	210 (62)
GR biotype	0.6 (0.3)	0.0 (0.0)	79 (5)	323 (75)	3,869 (676)	1.1 (0.2)	-1.9 (1.5)	93 (12)	306 (61)	2,022 (108)
Resistance level	-	-	-	-	3.0	-	-	-	-	1.6
R/S ratio	-	-	-	7.2	3.1	-			7.8	9.6
Giant Ragweed										
2,4-D	1.0 (0.7)	-0.6 (0.1)	80 (1)	15 (2)	277 (81)	1.4 (0.9)	-0.4 (0.2)	92	25 (5)	94 (42)
Glyphosate										
GS biotype	1.1 (0.1)	-0.6 (0.4)	87 (1)	59 (12)	956 (113)	1.5 (0.2)	-0.1 (0.0)	93 (2)	49 (16)	154 (67)
GR biotype	1.1 (0.2)	-1.0 (0.6)	79 (3)	349 (71)	5,879 (1,945)	1.2 (0.3)	-0.7 (0.5)	88 (2)	218 (44)	2,293 (621)
Resistance level	-	-	-	-	4.7	-	-	-	-	1.8
R/S ratio	-	-	-	5.9	6.1	-	-	-	4.4	14.9

[a] Abbreviations: d/n, day/night temperatures; ED_{50}, effective 2,4-D or glyphosate dose required for 50% control of common or giant ragweed; ED_{90}, effective 2,4-D or glyphosate dose required for 90% control of common or giant ragweed; SE, standard error.

[b] Regression parameters S (slope), L (lower limit) and U (upper limit) of the four-parameter log-logistic model ($Y = \mathrm{L} + \{\mathrm{U} - \mathrm{L}/1 + \exp[\mathrm{S}(\log\mathrm{X} - \log\mathrm{E})]\}$) were determined by using the nonlinear least-square function of the statistical software R.

[c] The resistance level was determined compared to the field rate of glyphosate (i.e., 1,260 g ae ha^{-1}) because the glyphosate-susceptible (GS) biotypes became too sensitive at the high temperature regime, leading to instability in the resistance level determined on the basis of the R/S ratio.

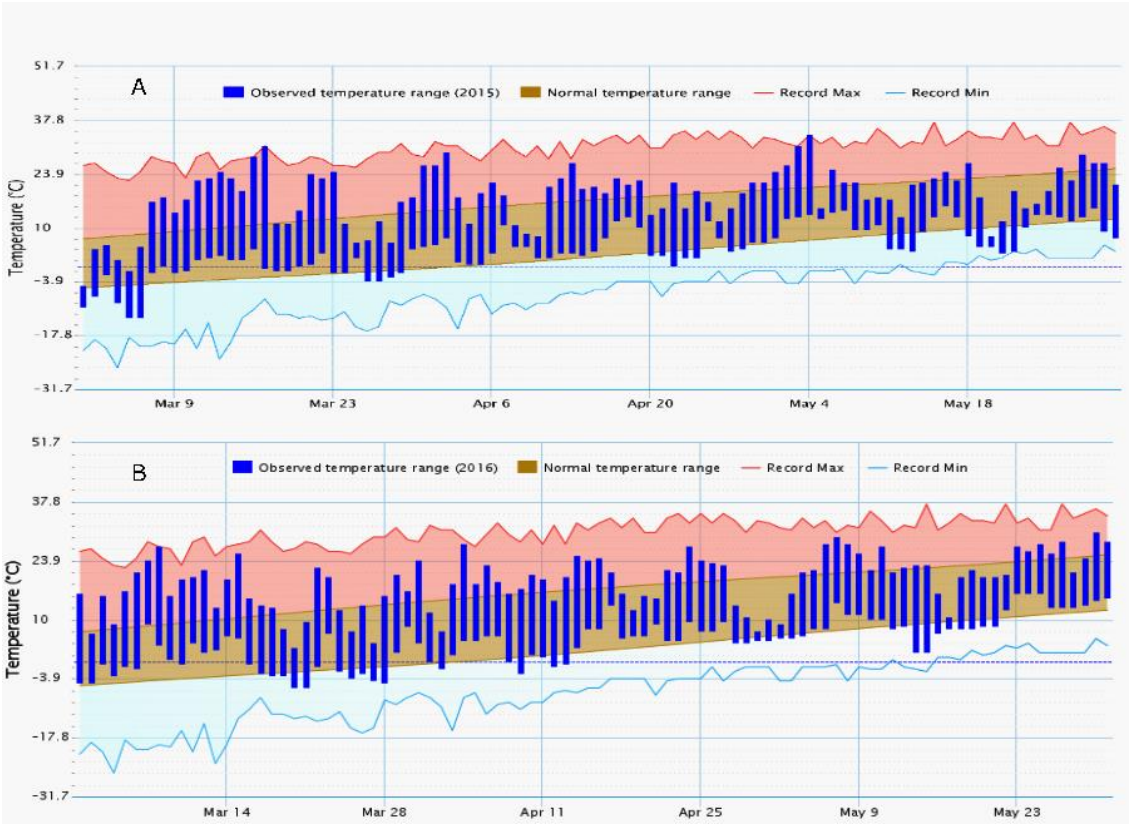

Figure 3.1. Daily average air temperature (^{0}C) from March to May in 2015 (A) and 2016 (B) and the normal temperature range [30 year average (1981 to 2010)] in south-central Nebraska. Weather data were obtained from the High Plains Regional Climate Center (HPRCC; http://www.hprcc.unl.edu).

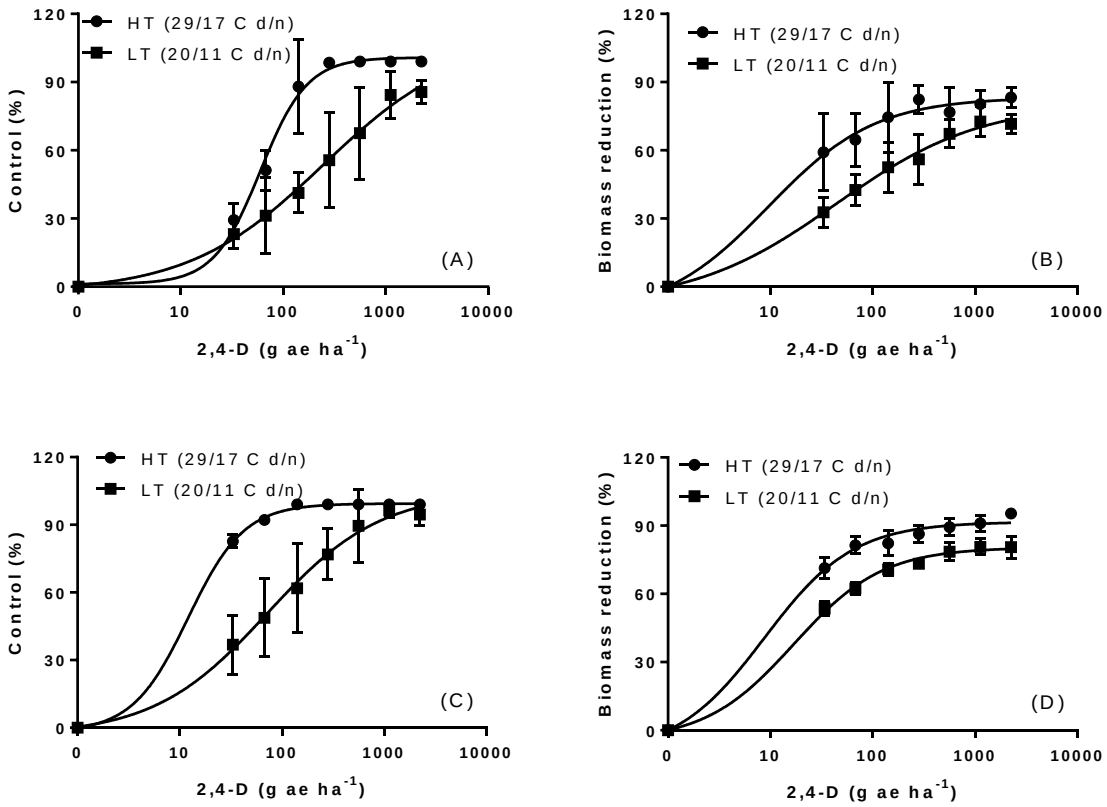

Figure 3.2. Dose-response curves of common ragweed and giant ragweed to 2,4-D applied at high and low temperature regimes at 21 d after treatment; (A) control of common ragweed, (B) biomass reduction of common ragweed, (C) control of giant ragweed, and (D) biomass reduction of giant ragweed.

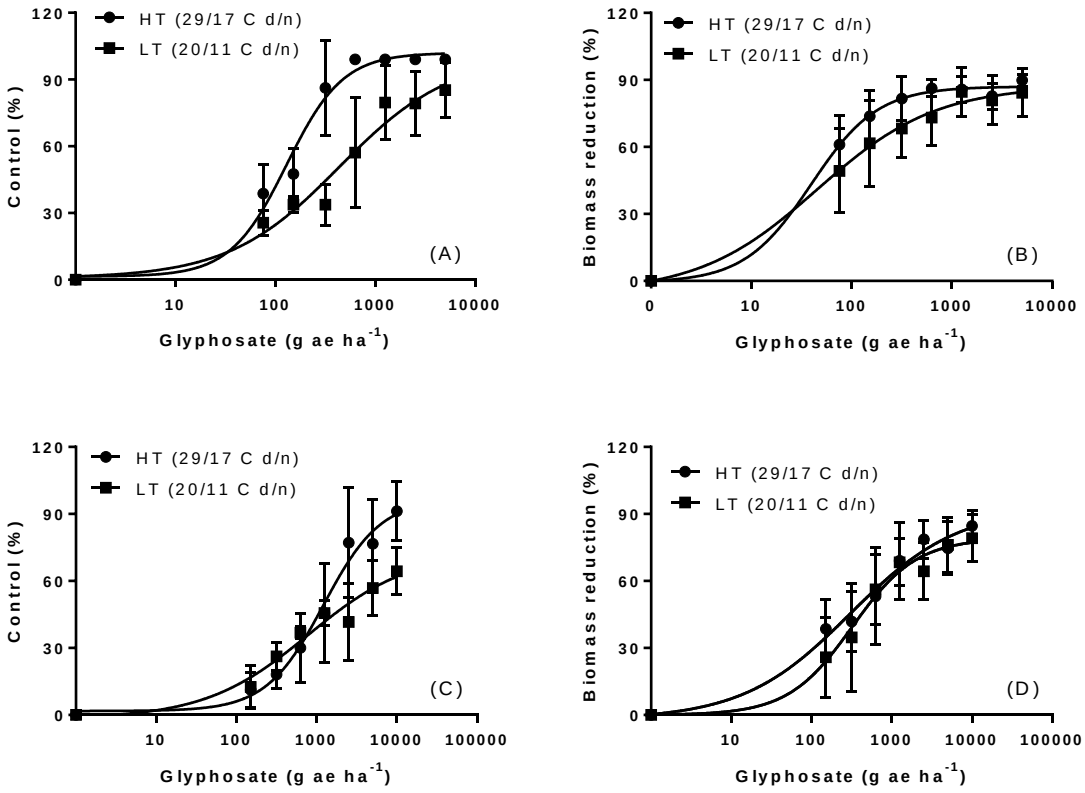

Figure 3.3. Dose-response curves of glyphosate-susceptible (GS) and –resistant (GR) common ragweed biotypes to glyphosate applied at high and low temperature regimes at 21 d after treatment; (A) control of GS common ragweed, (B) biomass reduction of GS common ragweed, (C) control of GR common ragweed, and (D) biomass reduction of GR common ragweed.

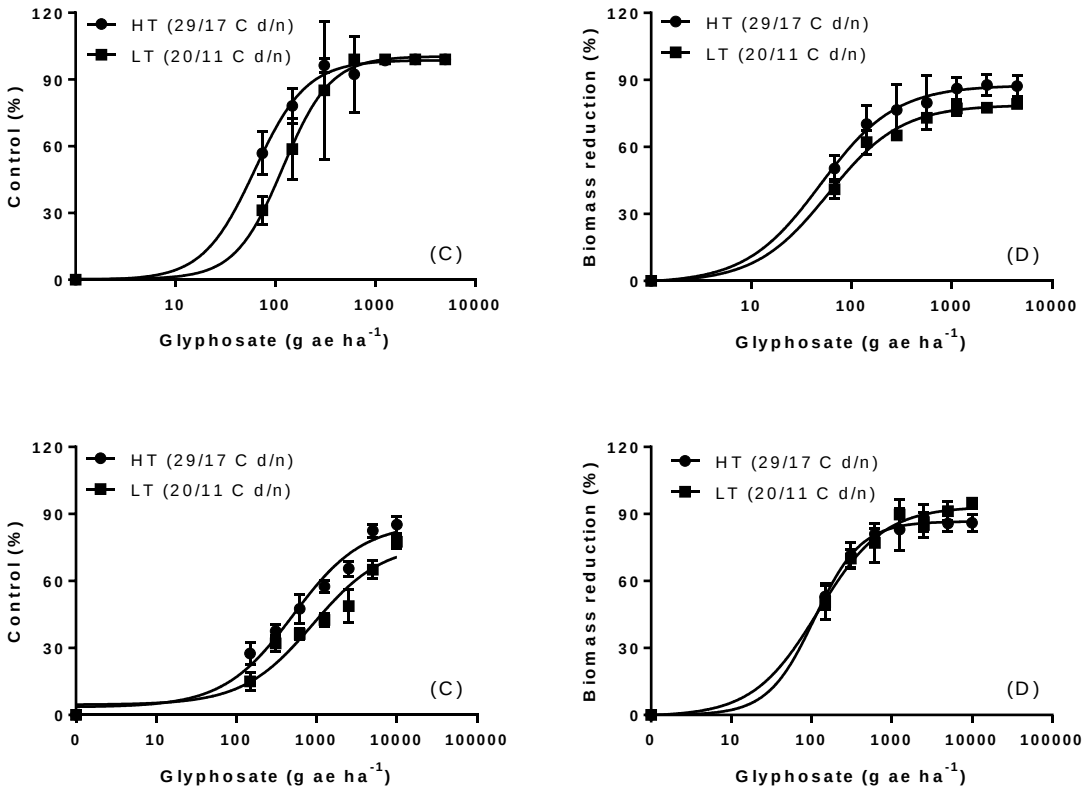

Figure 3.4. Dose-response curves of glyphosate-susceptible (GS) and –resistant (GR) giant ragweed biotypes to glyphosate applied at high and low temperature regimes at 21 d after treatment; (A) control of GS giant ragweed, (B) biomass reduction of GS giant ragweed, (C) control of GR giant ragweed, and (D) biomass reduction of GR giant ragweed.

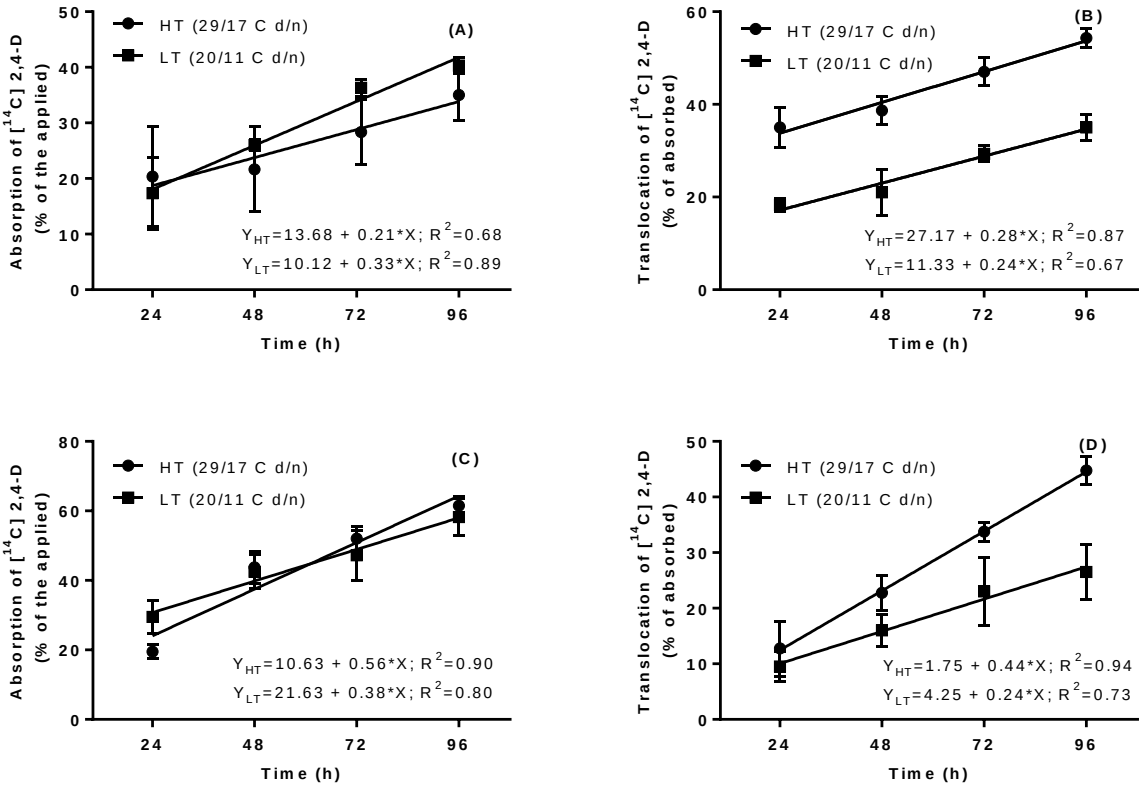

Figure 3.5. Absorption and translocation of 2,4-D in common and giant ragweed over time at two temperature regimes; (A) 2,4-D absorption in common ragweed, (B) 2,4-D translocation in common ragweed, (C) 2,4-D absorption in giant ragweed, and (D) 2,4-D translocation in giant ragweed.

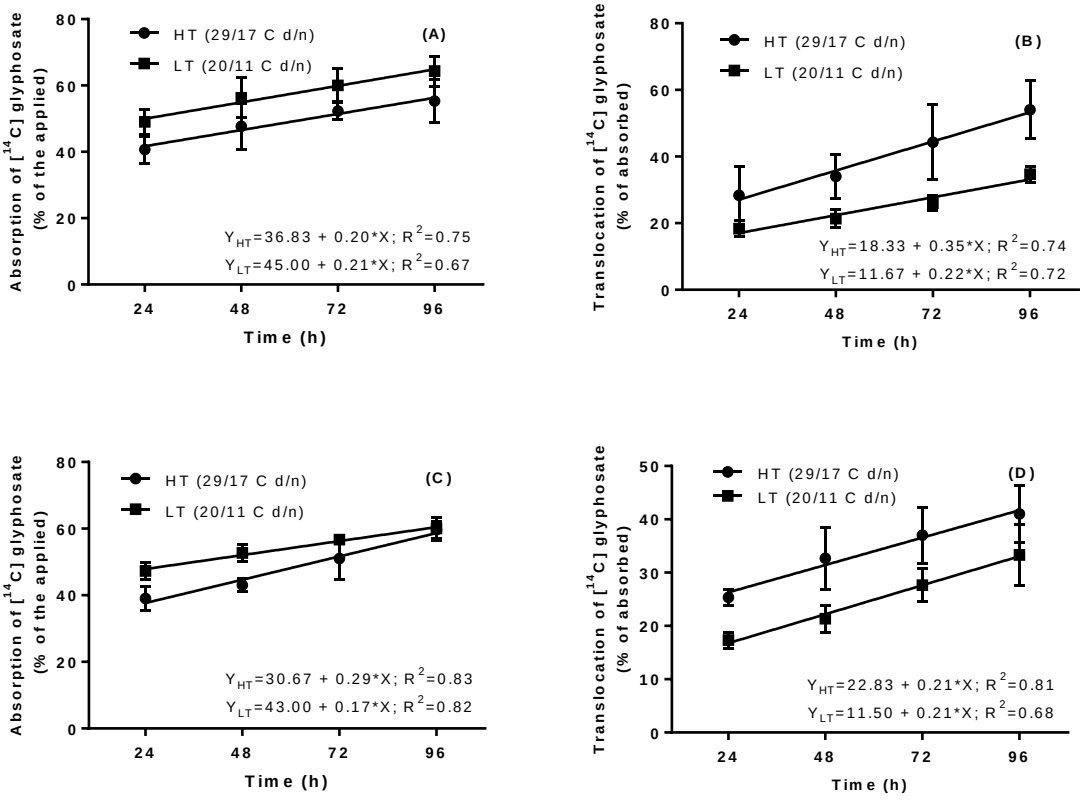

Figure 3.6. Absorption and translocation of glyphosate in glyphosate-susceptible (GS) and –resistant (GR) common ragweed over time at two temperature regimes; (A) glyphosate absorption in GS common ragweed, (B) glyphosate translocation in GS common ragweed, (C) glyphosate absorption in GR common ragweed, and (D) glyphosate translocation in GR common ragweed.

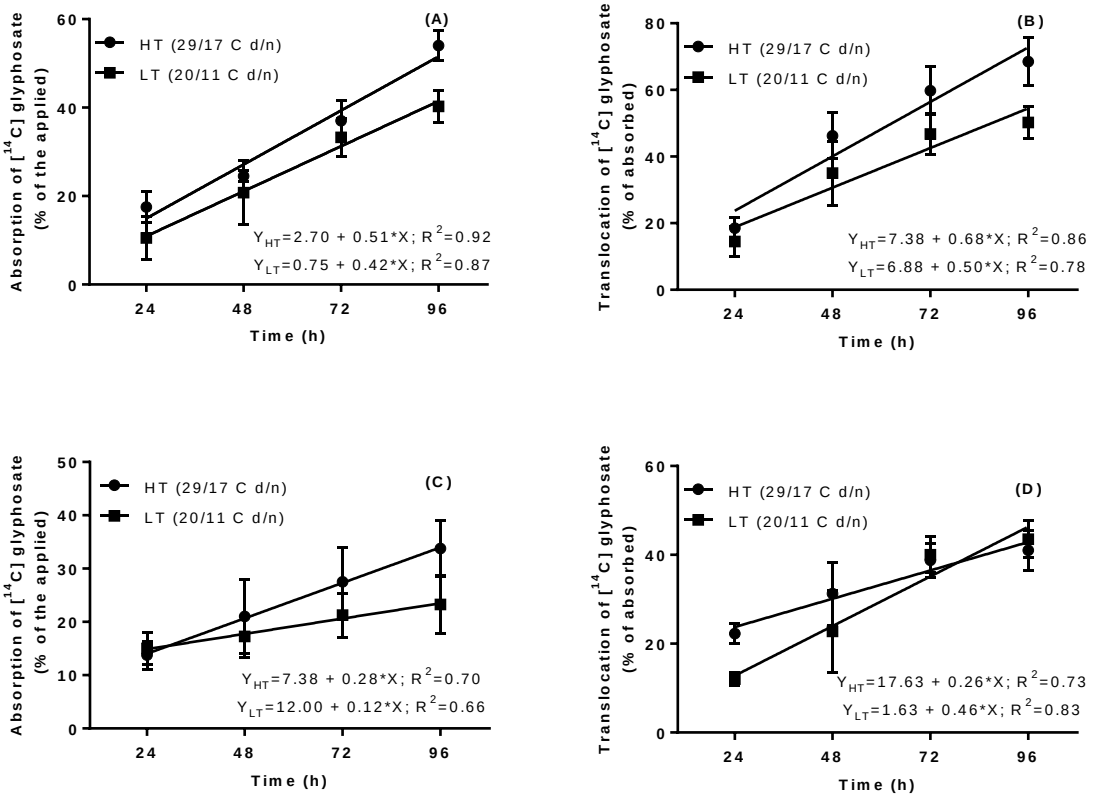

Figure 3.7. Absorption and translocation of glyphosate in glyphosate-susceptible (GS) and –resistant (GR) giant ragweed over time at two temperature regimes; (A) glyphosate absorption in GS giant ragweed, (B) glyphosate translocation in GS giant ragweed, (C) glyphosate absorption in GR giant ragweed, and (D) glyphosate translocation in GR giant ragweed.

CHAPTER 4: POLLEN-MEDIATED GENE FLOW FROM GLYPHOSATE-RESISTANT TO -SUSCEPTIBLE GIANT RAGWEED (*Ambrosia trifida*) UNDER FIELD CONDITIONS

Abstract

Widespread occurrence of glyphosate-resistant (GR) weeds in the Midwestern United States, especially species with certain degree of open pollination, have warranted to determine the role of pollen mediated gene flow (PMGF) in dispersal of resistance genes. Field experiments were conducted in 2014 and 2015 at the South Central Agricultural Laboratory (SCAL), Clay Center, NE to quantify PMGF from GR to –susceptible (GS) giant ragweed under non-crop field conditions using GR phenotype as a selective marker. The experiments were conducted by using a modified Nelder wheel design with the pollen source (GR giant ragweed) planted in the center and the pollen receptors (GS giant ragweed) planted surrounding the center in eight directional blocks (cardinal: N, S, E, and W; ordinal: NE, NW, SE, and SW) at specified distances (0.1, 0.5, 1, 2, 4, 10, 15, 25, and 35 m for all cardinal and ordinal directions; and additional 50 m for the ordinal directions) from the pollen source. Seeds were harvested from the pollen receptor blocks from all distances and a total of 98,967 giant ragweed plants were screened with 2× (× = 1,260 g ae ha^{-1}) rate of glyphosate and 17,367 plants were confirmed resistant to glyphosate. The frequency of PMGF from all the distances and directions were fit to a double exponential decay model selected by information-theoretic criteria using Generalized Nonlinear Model (*gnm*) library in R software. The highest frequency of gene

flow (0.43 to 0.68) was observed at ≤ 0.5 m distance from the pollen-source and the frequency of gene flow reduced rapidly with increasing distances from the pollen source; however, gene flow (< 0.05) was detected even up to 50 m distance, the highest distance evaluated in this study. Wind parameters (wind speed, wind direction, and wind frequency) were positively correlated with PMGF. Averaged across all directions, PMGF reduced by 50% (O_{50}) at ≤ 7 m distance from the pollen source, whereas 90% reduction (O_{90}) occurred at < 107 m distance. The results of this study are important to understand the reproductive biology of giant ragweed and confirmed that PMGF is an important means for dispersal of resistance genes in this species.

Introduction

Gene flow is the natural process of dissemination of genetic information from one breeding population to another (usually) related population (Glover 2002). More precisely, gene flow includes the movement of genes between individuals leading to the incorporation of new genes into the gene pool of another population (Futuyma 1998), or change in the frequency of existing genes in a population (Glover, 2002; Mallory-Smith and Zapiola 2008). Pollen-mediated gene flow (PMGF) is the movement of genes via pollen within and between populations of the species of the same genetic background (Manasse 1992). PMGF occurs in almost all flowering plant species due to the movement of pollen through wind, water, and pollinators (Ellstrand et al. 1999; Glover 2002; Mallory-Smith and Zapiola 2008). The frequency of PMGF depends on several factors, including reproductive biology, breeding system, pollen viability, pollen dispersal mechanism of a plant species, etc. (Loveless and Hamrick, 1984; Mallory-Smith et al.

2015). Furthermore, size, structure, and proximity among the populations (Ennos 1994; Heywood 1991) and environmental factors also play a role in PMGF (Iñigo Loureiro et al. 2016; Schmidt et al. 2013). Gene flow is considered a strong and dynamic evolutionary force that promotes evolution and speciation along with natural selection and influences the genetic diversity, adaptation, and fitness in a population (Ehrlich and Raven 1969; Ellstrand 2003; Gressel 2015). On the contrary, in the absence of natural selection and genetic drift, gene flow promotes genetic homogeneity and maintains genetic cohesiveness in a population (Délye et al. 2010; Ellstrand et al. 1999; Slatkin 1987).

Concerns related to gene flow in agriculture prominently became highlighted in media and scientific literature due to the development and commercialization of genetically-modified (GM) crops raising questions about the co-existence of GM and conventional/organic (non-GM) crops (Mallory-Smith and Zapiola 2008; Jhala et al. 2011). The major concern with transgenic crops is escape of the transgene into non-transgenic (conventional or organic) crops or to closely related species (Ellstrand 1988; Jhala et al. 2008; Légère 2005; Kuvshinov et al. 2001; Warwick et al. 2003; Watrud et al. 2004;). Additional concerns with transgenic crops include emergence of volunteers as weeds in subsequent crops, such as glyphosate-resistant (GR) corn volunteers in GR soybean fields in the Midwest (Chahal and Jhala 2015) and evolution of new invasive plants in the natural habitats (Crawley et al. 1993). A rapid adoption of the GM-crops occurred with the commercialization of GR crops including soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), canola (*Brassica napus* L.) and cotton (*Gossypium hirsutum*

L.) (Cerdeira and Duke 2006; Owen and Zelaya 2005; Reddy, 2001; Gianessi, 2005). GR crops revolutionized weed management by permitting in-crop use of glyphosate—a once in a century herbicide (Duke and Powles 2008). Glyphosate is unique due to its effectiveness on a wide-spectrum of grasses and broadleaf weeds and has relatively marginal detrimental effect on the environment (Cerdeira and Duke 2006; Duke and Powles 2008). The success of GR crops encouraged the use of conservation tillage resulting in a considerable increase in the profitability of agronomic cropping systems (Gianessi, 2005). However, glyphosate became a victim of its own success and rather than adding a new mode of action to the list of available herbicides, glyphosate use has reduced the diversity of herbicides used for weed control (Shaner et al. 2012; Young 2006).

The overreliance on glyphosate use for weed control in GR crops resulted in the evolution of glyphosate resistance in several grass and broadleaf weeds (Heap 2016). As of September 2016, 35 weed species including 16 grasses and 19 broadleaf weeds have evolved resistance to glyphosate worldwide, including 16 species in the United States (Heap 2016). The evolution of GR weeds not only reduces weed control options and utility of GR crops but also has long-term ecological consequences such as the persistence of the resistance trait in agricultural ecosystems and shifts in weed species composition. In most of the weed species, the evolution of glyphosate resistance is due to target and/or non-target site mechanisms, controlled by a single dominant or semi-dominant gene with nuclear inheritance (Jasieniuk et al. 1996; Powles and Preston 2006). Thus, the chances for spread of glyphosate resistance through the pollen movement is

possible, especially in cross-pollinated species (Jasieniuk et al. 1996). Gene flow via pollen dispersal delivers an initial source of resistance alleles in a susceptible weed population at a higher rate compared to the hypothetical mutation rate (1×10^{-6} for a gamete at a locus per generation) resulting in a rapid evolution and dissemination of resistance genes in new areas (Ellstrand 2003; Jasieniuk et al. 1996).

PMGF from GM crops to conventional crops or their weedy and wild relatives has been extensively studied to understand the consequences of domesticated alleles or transgenes in natural populations (Abud et al. 2007; Beckie et al. 2003; Cantamutto and Poverene 2007; Darmency et al. 2007; Devaux et al. 2005; Ellstrand et al. 1999; Ferreira et al. 2007; Goggi et al. 2007; Gustafson et al. 2006; Hall et al. 2000; Jia et al. 2007; Kuroda et al. 2005; Lim et al. 2007; Llardi and Barba 2001; Rieger et al. 2002; Rong et al. 2007; Saeglitz et al. 2000; Schmidt et al. 2013; Schmidt and Bothma 2007; Shivrain et al. 2007; Ureta et al. 2007; Wang et al. 2004; Youshimura 2006); however, limited literature is available on the dissemination of herbicide resistant traits between weed biotypes of the same species or closely related weed species (Bagavathiannan and Norsworthy 2014; Busi et al. 2008; Fénart et al. 2007; Murray et al. 2002; Sosnoskie et al. 2012; Stallings et al. 1995; Yerka et al. 2012).

The rapid evolution of GR weeds and their widespread occurrence has warranted a need to evaluate the spread of the resistant traits under natural conditions. The fate of a resistant allele in a weed population is influenced by the frequency, number, dominance and heritability of the resistance genes, fitness cost, and reproductive and gene dispersal systems of the resistant biotype (Jasieniuk et al. 1996; Powles and Yu 2010, Roush et al.

1990). PMGF is particularly important in weed species characterized with outcrossing nature and restricted seed mobility due to large seed size such as giant ragweed (Brabham et al. 2011). Giant ragweed is a competitive summer annual broadleaf weed found throughout the United States and southern Canada (Abul-Fatih and Bazaz 1980; Bassett and Crompton 1982; Hunt and Bazzaz 1980). Giant ragweed is a monoecious species meaning that separate male and female flowers are present on the same plant. The male flowers occur in the terminal recemes at the top of the plant and female flowers are found in clusters at axils below male flowers (Johnson et al. 2006). The male flower produces considerably more pollen grains than the female flowers need to pollinate on a single plant. During flowering, a single giant ragweed plant can produce an estimated 10 million pollen grains daily and more than a billion pollen grains during its life cycle (Johnson et al. 2006). The exposure to giant ragweed pollen causes allergic rhinitis and asthma (Ziska et al. 2011). Excessive pollen production allows giant ragweed plants to cross-pollinate, leading to much variation in physical appearance and genetic diversity and consequently, a greater potential for resistance genes to migrate through pollen movement (Johnson et al. 2006). Abundant pollen production, wind-pollination with a potential long-distance dispersal of pollen up to about a kilometer (Raynor et al. 1970), and outcrossing nature of giant ragweed increase the chances of PMGF between giant ragweed biotypes.

Studies on the pollen dispersal characteristics of giant ragweed using acetolactate synthase (ALS)-resistant biotypes suggested that the majority of pollen remained within 5 m and declined rapidly as the distance from pollen-source increased (Volenberg et al. 2005). Brabham et al. (2011) documented an outcrossing rate of 31% between GR and

glyphosate-susceptible (GS) giant ragweed biotypes at a distance of 76 cm (row spacing) and also suggested that GR is expressed as a dominant phenotype in giant ragweed. However, scientific literature is not available on the spread of glyphosate resistance in giant ragweed in the current glyphosate-dominated agricultural cropping systems. Therefore, the objective of this study was to determine the PMGF between GR and GS giant ragweed biotypes under natural, non-crop situation, and to understand the potential role of physical distance, wind speed and direction in the dissemination of glyphosate resistance trait in giant ragweed.

Materials and Methods

Plant Material. Seed heads of the GR giant ragweed biotype were collected in 2013 from a grower's field near David City (41.26^{0}N, 97.14^{0}W), NE. The level of glyphosate-resistance in this biotype was 14-fold compared with a known susceptible biotype (Rana et al. 2013). Similarly, seed heads were collected from a known GS giant ragweed biotype from the South Central Agricultural Laboratory (SCAL), University of Nebraska-Lincoln near Clay Center (40.58^{0}N, 98.14^{0}W), NE. Seed heads were manually threshed using a hand-held roller and cleaned using a seed blower (South Dakota Seed Blower, Seedburo Equipment Co., 1022 W. Jackson Blvd., Chicago, IL). To overcome dormancy, giant ragweed seeds were packed in mesh bags, placed between the moistened soil layers in plastic boxes and then stored in deep freezer at -8 C for 3.5 months before using in this study (Kaur et al. 2016).

Seeds from the GR and GS biotypes were germinated in 72-celled plastic germination trays containing potting mix (Berger BM1 All-Purpose Mix, Berger Peat

Moss Ltd., Saint-Modeste, Quebec, Canada). One plant per cell was maintained after two weeks and extra plants were transplanted to additional germination trays to raise vigorous seedlings for transplanting. Plants were maintained in the greenhouse with a daytime temperature of 25 ± 2 C and a nighttime temperature of 18 ± 3 C, and a relative humidity of 70 to 75%. Sodium halide lamps were used as a supplemental light source to ensure a 15-h photoperiod. Plants were supplied with adequate nutrients as needed and watered daily, except in the week before transplanting when water was added alternately to acclimatize the plants. Glyphosate resistance and susceptibility of the GR and GS biotypes was further verified in both years by treating a randomly selected sample of 100 plants from each biotype with 1× (1,260 g ae ha^{-1}) rate of glyphosate (Touchdown HiTech®, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC) (Figure 4.1). The seedlings of both the biotypes were transplanted to the field when majority of plants attained 8 to 12 cm height.

Field Experiments. A field experiment was conducted in 2014 and 2015 at the University of Nebraska-Lincoln, South Central Agricultural Laboratory (SCAL) at Clay Center (40.58^0N, 98.14^0W). The soil texture at the experimental site was a Crete silt loam (fine, montmorillonitic, mesic, Pachic Argiustolls) consisting of 17% sand, 58% silt, 25% clay, 2.5% organic matter, and a pH of 6.5. The primary weed species observed at the experimental site were common lambsquarters (*Chenopodium album* L.), common waterhemp (*Amaranthus rudis* Sauer), green foxtail [*Setaria viridis* (L.) Beauv.], Palmer amaranth (*Amaranthus palmeri* S. Wats.), and velvetleaf (*Abutilon theophrasti* Medik.). There was no suspicion or report of any GR weed species on or around the experimental

site. Field preparation started early May with tillage using a tandem disk harrow followed by an application of micro-encapsulated acetochlor (1.68 kg ai ha^{-1}) (Warrant®, Monsanto Company, 800 N, Lindbergh Blvd., St. Louis, MO 63167) tank-mixed with glyphosate (0.87 kg ae ha^{-1}) (Roundup PowerMax®, Monsanto Company, 800 N, Lindbergh Blvd., St. Louis, MO 63167) to control early-season weeds. Later in the season, the experimental site and its surrounding area (up to 60 m) was kept weed free either by hand-weeding or cultivation. The experiments were conducted under a non-crop situation without any physical barriers to obstruct the natural wind or pollen movement.

The field experiments were conducted using a modified Nelder wheel design with the pollen source (GR giant ragweed) planted in the center and the pollen receptors (GS giant ragweed) planted around the center (Jhala et al. 2011; Nelder 1962; Walsh et al. 2015). GR giant ragweed biotype served as the pollen donor and the GS biotype served as the pollen receptor. The experimental area was 80 m × 80 m with a central circle of 80 sq m (10 m diam) for the pollen-donor block (Figure 4.3). Approximately 377 GR giant ragweed plants were transplanted in the pollen donor block in East-West and North-South directions in a grid pattern with 0.46 m plant to plant distance. The transplanting was performed on June 9 in 2014 and May 26 in 2015.

The receptor area was divided into eight directional blocks (cardinal: N, S, E, and W; ordinal: NE, SW, SE, and SW) and six plants of the GS biotype were transplanted with plant to plant spacing of 0.3 m at each of the specified distances (0.1, 0.5, 1, 2, 4, 10, 15, 25, 35 m for all cardinal and ordinal directions; and additional 50 m only for the ordinal directions) from the pollen-donor block (Figure 4.3).

Flowering Period and Seed Harvesting. The percentage of flowering plants was noted at 5 d intervals for the pollen-donor and -receptor blocks and flowering synchrony was evaluated for each direction using the equation (Sarangi 2016):

$$\textit{Flowering synchrony}\ (\%) = \frac{1}{n}\sum_{i}^{n}\frac{A_i\%}{B\%} \times 100 \qquad [1]$$

where, n is the total number of distances in each direction, $A_i\%$ is the percentage of flowering plants at the *ith* observation (distance) in the pollen-receptor blocks, and $B\%$ is the percentage of plants shedding pollen in the pollen-donor area at that time. $A \geq B$ means fully synchronized flowering (i.e. 100%) in the pollen receptor.

At maturity, the seedheads of GS giant ragweed plants from each distance and direction were hand-harvested, bagged, and separately labeled. The seeds were harvested and cleaned thoroughly, and stratified to break seed dormancy by the same procedure described earlier.

Meteorological Data. Hourly surface meteorological data were recorded by the Bowen ratio energy balance systems (BREBS) stations of the Nebraska Water and Energy Flux Measurement, Modeling, and Research Network (NEBFLUX) available at the South Central Agricultural Laboratory, Clay Center, NE (Irmak 2010). Wind frequency (frequency of time during which the wind blows towards a certain direction), wind speed, and wind run (calculated by multiplying the average wind speed by the wind frequency; Schmidt et al. 2013) data were used for modeling PMGF, whereas other meteorological data such as temperature, humidity, and precipitation were recorded due to their effect on pollen viability and dispersal (Shivanna et al. 1991).

Resistance Screening. Greenhouse dose-response bioassays for the parent biotypes (GR and GS) were conducted and the effective doses of glyphosate required for 50 (ED_{50}) and 90% (ED_{90}) injury of the parent biotypes were determined using the *drc* package in R software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria) (Knezevic et al. 2007). The ED_{50} values for the GR and GS biotypes were 1,722 and 112 g ae ha^{-1}, respectively, whereas the ED_{90} values were 14,254 and 468 g ae ha^{-1}, respectively (Figure 4.2).

Seeds collected from the GS giant ragweed plants were germinated separately for each distance and direction in the greenhouse and evaluated for glyphosate resistance. Plastic trays (51 cm × 38 cm × 10 cm) containing potting mix (described previously) were used for growing the plants. A maximum of 130 plants were allowed per tray to ensure sufficient glyphosate coverage on the leaf surface. The putative hybrid plants were sprayed at an 8-10 cm height with 2× the recommended rate of glyphosate (Touchdown HiTech®, Syngenta Crop Protection, LLC, P.O. Box 18300, Greensboro, NC 27419-8300), where 1× = 1,260 g ae ha^{-1}. The resistance screening was performed at the 2× rate (2,520 g ae ha^{-1}) of glyphosate to obtain more consistency in the response of giant ragweed plants to glyphosate with complete mortality of all the susceptible plants present, and to assure the survival of any GR plant (as the ED_{90} value for GR parent plants was 5.6-times higher than the 2× rate of glyphosate). The number of seedlings surviving glyphosate treatment were recorded at 21 d after application and the frequency of gene flow at each distance/direction was calculated using equation:

$$Frequency\ of\ gene\ flow = \frac{Number\ of\ surviving\ plants}{Number\ of\ sprayed\ plants} \qquad [2]$$

Statistical Analysis. Information-theoretic approach (Burnham and Anderson 1998, 2002; Taper 2004) of model selection was used to select the best model for analyzing the PMGF between GR and GS giant ragweed. Unlike traditional null hypothesis testing, the model selection approach allows simultaneous evaluation of multiple competing hypotheses (models) rather than only two (the null and a single alternative hypothesis) (Johnson and Omland 2004; Taper 2004).

Usually the frequency of gene flow follows a binomial distribution, and the two possible outcomes in this study were either dead (susceptible) or live (resistant) giant ragweed seedlings after screening with glyphosate. A characteristic of binomial distribution is that mean and variance are equal and dependent on the underlying probability function, p_i. A set of possible models were generated to explain the frequency of PMGF using an exponential decay function with distance from the pollen source, direction of the pollen-receptor blocks, average wind speed, wind frequency, and/or wind run as the explanatory variables in different logically possible combinations without collinearity. A set of 43 total possible models were constructed, though few non-convergence iterations were also observed due to the complexity of the models (Van der Elst et al. 2015). The nonlinear regression models were fit using the Generalized Nonlinear Models (gnm) package in R software. The advantages of using the gnm compared to the nonlinear least square (*nls*) function includes that responses with non-Gaussian distribution can be fitted, and its convenience to represent a model with a large number of parameters by symbolic model specification (Turner and Firth 2015).

Model Selection. The Akaike's Information Criterion (AIC) was followed for comparison of the candidate models and selection of the best model using the equation (Anderson 2010)

$$AIC = -2LL + 2K \qquad [3]$$

Where, LL is the log-likelihood function for the models, and K is the number of parameters estimated. The lower the AIC value, the better the model; therefore the model with lowest AIC value was considered as the best candidate model (Collett 2003).

Best Model. The best model describing this data was selected based on the model selection criteria including AIC, $delta - AIC$, and LL. The best fit to data was provided by a double exponential decay model (Equation 4) where frequency of the PMGF varied with the distance from the pollen source, the direction of the pollen-receptors, and the year.

$$logit(p_i) = \beta_0 + \exp[\beta_1 + \gamma_1 \times distance] + \exp[\beta_2(direction{:}\,year) + \gamma_2(direction{:}\,year) \times distance + \varepsilon \qquad [4]$$

where p_i is the frequency of gene flow of the i^{th} observation; β_0 is overall intercept; β_1, β_2 are the intercepts for the first and second instances, respectively; γ_1, γ_2 are the decay rates where $\gamma_1 > \gamma_2$ and ε is the error term. Here, β_2 and γ_2 vary with the direction and the year.

In binomial distribution, probability (p_i) is the function of the covariate ($\gamma_i x_i$) (x is the distance from pollen source) that can take any real value. The p_i ranges between 0 and 1 ($0 \leq p_i \leq 1$). Therefore, transformation of the probability becomes important to remove the range and floor restrictions. $Logit$, or log-odds were calculated using the

transformation methods described by Cramer (2003), whereas the back-transformed data were presented:

$$Logit, \eta_i = logit\ (p_i) = \ln(\frac{p_i}{1-p_i})$$

$$Back\ transformation, p_i = logit^{-1}(\eta_i) = \frac{e^{\eta_i}}{1+e^{\eta_i}} \qquad [5]$$

The distance where the frequency of gene flow was reduced by 50% (O_{50}) and 90% (O_{90}) of the frequency predicted at closest distance were estimated from the final model (Equation 4).

Model Goodness of Fit. Goodness of fit statistic was estimated for the best model by measuring the difference between observed and fitted values. Model goodness of fit was determined by $Pearson's\ chi-squared\ statistic$, which can be written for binomial data as [Equation 6]

$$\chi^2{}_{(n-k-1)} = \sum_i \frac{n_i(y_i-\hat{\mu}_i)^2}{\hat{\mu}_i(n_i-\hat{\mu}_i)} \qquad [6]$$

where the sum of squared differences between y_i (observed values) and $\hat{\mu}_i$ (fitted values for the i^{th} group of observations) was divided by the variance of y_i that was $\mu_i(n_i-\mu_i)/n_i$ (with μ_i estimated using $\hat{\mu}_i$) and n_i is the sample size for i^{th} group. The degree of freedom for Pearson's chi-squared statistic was $n-k-1$, where n refers to the total number of groups and k was the number of parameters.

Results and Discussion

Flowering Synchrony. Giant ragweed is a protogynous species, meaning that female flowers become receptive before the male flowers start shedding the pollen (Bassett and Crompton 1982). Initiation of flowering was observed on July 25, 2014 and July 30,

2015, and the protrusion of stigmas in the female flowers occurred on average 3 to 5 days ahead of the pollen shed from the male flowers on the same plant (Table 4.1). GR giant ragweed plants in the center and GS plants within 4 m distance from the pollen source in different directions flowered together while the flowering was delayed by 3 to 6 days in GS plants at distances ≥ 10 m from the pollen source, possibly due to lower plant density and minimal competition for resources resulting in vigorous vegetative growth and a delay in the transition to reproductive phase. Peak flowering occurred 3 wk after floral initiation. However, continuous pollen production and a small number of new female flowers were observed until mid-September (Table 4.1). Bassett and Crompton (1982) reported that giant ragweed starts flowering in mid-July and continues until late August or early September in Canada. The total flowering period lasted 5 to 6 wks with a flowering synchrony of ≥ 80% between GR and GS giant ragweed biotype in both years (Table 4.1).

Meteorological Data. Mean daily temperature during the flowering period varied from 17 C to 31 C in 2014 and 20 to 30 C in 2015 (Figure 4.4). The average wind speed during the flowering period in 2014 and 2015 was 1.4 and 2.5 m s^{-1}, respectively. However, the pattern of wind flow remained similar in both years and most of the time wind blew from the south (S) or southeast (SE) (Figure 4.5). A positive correlation was observed in overall gene flow with wind speed, wind frequency, and wind run (Table 4.2 and 4.3). The correlation between the frequencies of gene flow and wind speed was significant up to 25 m distance from the edge of the pollen-source in each year with correlation coefficients (*r*) varying from 0.12 to 0.42 (Table 4.2 and 4.3). However, significant

correlation coefficients for wind frequency or wind run were observed only up to 10 m distance in 2014 and up to 15 m distance in 2015 (Table 4.2 and 4.3). High temporal variation in wind frequency or wind run and wind gusts may be the reason for absence of strong correlation with frequency of PMGF. Besides, giant ragweed is a monoecious species; therefore, competition between the GR pollen and locally available GS pollen from the pollen receptor area for successful events of fertilization are expected. The interactions of distance × direction within a year, and distance × direction × year were significant ($P < 0.05$) suggesting that the frequency of gene flow varied between the directions each year, and between the years at specified distances.

Frequency of Gene Flow. A sampling strategy suggested by Jhala et al. (2011) was followed to select the appropriate sample size for screening giant ragweed plants to quantify the PMGF with a power of ≥ 0.8. A total of 98,967 giant ragweed plants were screened in greenhouse and 17,367 plants were found resistant to glyphosate (Tables 4.4 and 4.5).

The frequency of gene flow declined with increasing distance from the pollen source following a leptokurtic pattern, though the magnitude varied between directions and years (Table 4.4 and 4.5). The highest frequency of gene flow averaged over eight directions was 0.6 to 0.68 (i.e., 60 to 68%) at ≤ 1 m distance in 2014 compared to 0.43 (43%) at 0.1 m distance from the edge of the pollen-donor block in 2015 (Table 4.4 and 4.5). PMGF reported in this study is relatively greater than the 31% gene flow between GR and GS giant ragweed planted in rows at a distance of 0.76 m reported by Brabham et al. (2011). The average frequency of gene flow declined to < 0.08 and ≤ 0.05 at 35 and 50 m

distance from the pollen source, respectively in both years. Raynor et al. (1972) reported that approximately 9% of the ragweed (*Ambrosia*) pollen released from the pollen source reached up to a distance of 60 m. A relatively high level of outcrossing in giant ragweed compared to other self-compatible species is likely due to its facultative outcrossing nature favored by anemophilous pollination and massive pollen production (Johnson et al. 2006).

A comprehensive model selection approach using Akaike's Information Criteria (*AIC*) was adopted to select the most parsimonious model to explain the frequency of gene flow as a function of the relevant explanatory variables without redundancy. A double exponential decay model (Equation 4) with distance, direction, and interaction of directions with the years was selected as the best model out of 43 candidate models based on *AIC* and *LL* (Tables 4.6 and 4.7). Similarly, Sarangi (2016) and Bagavathiannan and Norsworthy (2014) used a double exponential decay model to describe PMGF in common waterhemp (*Amaranthus rudis* Sauer) and barnyardgrass [(*Echinochloa crus-galli* (L.) Beauv.], respectively. All the top competitive models suggested that inclusion of direction as a covariate in modelling is more appropriate compared to the hourly wind data (wind speed, wind frequency or wind run), and the possible reason may be the huge temporal and spatial variation in the wind data (Table 4.8). Historically, very few PMGF studies included effect of direction in quantifying the frequency of gene flow (Sarangi 2016; Nurminiemi et al. 1998), which should be included to overcome the potential of over or under estimation of gene flow. Furthermore, the exponential decay curves also indicated that the frequency of gene flow varied in different directions in both years (Figures 4.6 and 4.7). Similarly, Beckie et al. (2016) reported the PMGF from GR to GS

kochia was influenced by wind direction. However, PMGF in common lamsquarters did not depend on the direction of wind (Yerka et al. 2012).

The predicted distances where gene flow was reduced by 50% (O_{50}) varied from 1.3 m to 7.0 m in 2014; and from 0.3 m to 2.4 m in 2015 (Table 4.6), depending on the direction with respect to the source. Similarly, the predicted distances for 90% (O_{90}) reduction in gene flow varied widely depending on the direction. However, the maximum distance at which 90% reduction in gene flow occurred was 49.5 m in the W arm in 2014 and 106.5 m in the N arm in 2015. Large confidence intervals of the predicted distances at which 90% reduction in gene flow occurred suggested a higher variability in frequency of gene flow at farther distances from the pollen source (Table 4.6). Volenberg et al. (2005) reported a percent gene flow of 31 and 5% at the distance of 5 and 60 m, respectively, from ALS-resistant to –susceptible giant ragweed. Similarly, several studies have documented that PMGF has a significant role in transferring and altering the frequency of resistance alleles within and between weed populations. For example, in a predominantly self-pollinated weed species such as common lambsquarters, PMGF varied from 3% at 2 m to 0.16% at 15 m from the pollen-source. Gene flow in giant foxtail (*Setaria faberi* Herrm.) ranged from 0.24 and 0.73% among plants grown 0.36 m apart (Volenberg and Stoltenberg 2002). In contrast, gene flow from imidazolinone-resistant domesticated sunflower (*Helianthus annuus* L.), a cross-pollinated species, to wild sunflower ranged from 11 to 22% and 0.3 to 5% at 2.5 and 30 m from pollen source, respectively (Massinga et al 2003). Recently, Beckie at al. (2016) reported 5.3 to 7.5%

gene flow at ≤ 1 m distance from GR to GS kochia and gene flow declined exponentially to 0.1 to 0.4% at 96 m distance.

The results suggested that PMGF has a significant role in the dispersal of GR alleles in giant ragweed causing an increase in the frequency of GR giant ragweed plants within the field populations, and has potential to introduce the GR alleles in nearby field or non-crop populations. Similarly, Sarangi (2016) reported PMGF from glyphosate-resistant common waterhemp to susceptible common waterhemp and potential spread of resistant alleles through pollen. In addition to gene flow, the dynamics of resistance in a population is determined by the initial frequency of the resistance alleles, heritability, reproduction, and fitness (Jasieniuk et al. 1996; Roush et al. 1990). Maxwell et al. (1990) identified two set of biological processes that influence the ecological fitness and gene flow as key factors in the evolution and dynamics of herbicide-resistant weed populations. Studies on the relative fitness of GR and GS giant ragweed reported contrasting results (Brabham et al. 2011; Glittner and Stoltenberg 2015). Brabham et al. (2011) reported that fitness penalty in a GR giant ragweed biotype from Indiana resulted in low fecundity in GR plants compared to GS plants, though the authors mentioned that different origin of the two biotypes might be the reason for differences in fecundity. In contrast, Glittner and Stoltenberg (2015) reported more fecundity and similar viability in the GR biotype compared to a GS biotype from Wisconsin in the absence of glyphosate. Walker et al. (2016) also reported that no fitness penalty is involved in GR giant ragweed biotypes from Mississippi, Ohio, and Tennessee compared to GS biotypes. In absence of fitness penalty, GR plants with greater fecundity will likely contribute higher proportions

of GR seeds into the soil-seed bank and the consequences will be an increased number of plants with GR trait in the giant ragweed population even in absence of glyphosate (Glittner and Stoltenberg 2015). Therefore, high frequency of PMGF and lack of fitness penalty in GR giant ragweed are ideal for wide spread occurrence of glyphosate-resistance in this species.

This is the first report of the long-distance dispersal of the GR alleles in giant ragweed and the results of this study are critical to explain wide spread occurrence of GR giant ragweed in the Midwest and may be useful in developing a simulation model to predict the spread of resistant alleles or the dissemination of multiple herbicide resistance alleles from the point of their origin. Pollen mediated gene flow enhances genetic variance in a population, increases the frequency of multiple or polygenic herbicide resistance, and the evolutionary dynamics of a species (Mallory-Smith et al. 2015). For example, two distinct GR phenotypes have been reported in giant ragweed, including GR biotypes with the rapid necrosis response and slow response biotypes (Brabham et al. 2011; Van Horn and Westra 2014), supporting the possibility that multiple mechanisms of resistance are involved (Van Horn and Westra 2016). However, a precise mechanism(s) of herbicide resistance in giant ragweed is still unknown─ though a partial role of altered translocation has been suggested (Nandula et al. 2015). It is possible that PMGF may bring rapid and slow response mechanisms together and result in the evolution of GR populations with more complex mechanism(s). Similarly, it is also possible that giant ragweed biotypes resistant to ALS-inhibitors and glyphosate might have evolved due to PMGF between them and more such cases should be expected in

future due to widespread occurrence of ALS and glyphosate-resistant giant ragweed in the Midwest. In a recent survey, Regnier et al. (2016) reported that herbicide resistance to ALS-inhibitors and glyphosate in giant ragweed were concentrated in the same counties and clusters of counties with multiple modes of resistance in Ohio, Indiana, Illinois, Missouri, Iowa, Nebraska and Minnesota. The same study reported that out of 15 states surveyed, the responses indicated that resistance to ALS-inhibitors, glyphosate and both (ALS + glyphosate) modes of action occurred in 13, 14 and 12 states in contrast to officially confirmed reports from 5, 11 and 3 states, respectively.

For pollen to be effective to fertilize over long distance gene dispersal, extended pollen viability is required (Dafni and Firmage 2000). The characteristics of the ragweed pollen including flattened to nearly spherical shape, presence of numerous spine-like projections on the surface, small pollen size varying from 18 to 25 µm and low velocity of deposition (0.02 to 0.06 m s^{-1}) likely favors long distance pollen dispersal (Barnes et al. 2001; Kanter et al. 2013; Pasqualini et al. 2011). However, information on the duration of pollen viability in giant ragweed is not available. Additionally, since this study was conducted under non-crop situation with a small pollen-source, the results may vary compared to field situations with crops or other weed species acting as vegetation barriers, and the ratio of GR to GS plants. Therefore, future studies should consider evaluating duration of pollen viability and landscape level dissemination of GR trait in giant ragweed.

Practical Implications of PMGF in Glyphosate-Resistant Giant Ragweed Management. Based on the results of this study it is evident that pollen-mediated

dissemination of the GR trait is possible in giant ragweed and it depends on multiple factors, including distance from the pollen source, wind speed, and wind direction. Therefore, necessary adjustment in the management approach are needed such as control of giant ragweed escapes before flowering, communication and collaboration among the growers to avoid farm to farm spread of GR. Awareness among the growers about the significance of PMGF in the spread of resistance genes from herbicide-resistant to susceptible weed species is needed (Bagavathiannan and Norsworthy 2014). The adoption of integrated weed management approaches with diversified strategies should be encouraged to avoid the widespread distribution of existing herbicide resistant traits as well as to delay the evolution of new herbicide resistant weeds (Ganie et al. 2016; Jhala et al. 2014).

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Table 4.1. Flowering synchrony between glyphosate-resistant and –susceptible giant ragweed in the pollen-mediated gene flow study conducted in 2014 and 2015.

Directions	Flowering synchrony*							
	2014				2015			
	Aug 1	Aug 10	Aug 25	Sep 10	Aug 1	Aug 15	Aug 30	Sep 10
N	1.4	1.5	1.0	0.9	1.5	1.0	1.1	0.8
S	0.6	1.3	1.1	1.4	0.8	1.2	1.0	0.9
E	0.9	1.3	1.0	1.6	1.7	1.0	1.0	1.0
W	1.5	1.2	1.0	1.2	1.3	1.0	1.1	1.5
NE	1.1	1.5	1.0	1.0	1.3	1.0	1.0	0.9
NW	1.4	1.5	1.0	1.0	1.6	1.1	1.1	1.1
SE	1.3	1.4	1.0	1.0	1.6	1.0	1.2	1.2
SW	1.5	1.4	1.0	1.0	1.4	1.3	1.0	1.0
Average	1.2	1.4	1.0	1.1	1.4	1.2	1.1	1.1
% flowering plants in pollen-donor block	25	45	99	65	35	60	99	60

* Flowering synchrony between glyphosate-resistant and –susceptible giant ragweed was calculated using Equation: $X_i = \frac{1}{n}\sum_{j=1}^{n}\frac{A\,\%}{B_j\,\%}$, where n is the total number of distances in direction i, A % is the percentage of plants shedding pollen in the pollen-donor area, and B_j % is the percentage of flowering plants at the j^{th} observation (distance) in the pollen-receptor blocks at that specific time. X = 1.0 means perfect synchrony between the pollen donor and the receptor. X > 1.0 shows that sufficient pollens from GR male plants were present to pollinate GS females, but X values as low as 0.5 was not considered a good synchrony.

Table 4.2. Pearson correlation coefficients (*r*) between wind parameters (wind speed, wind frequency, and wind run) and frequency of gene flow at different distances.[a, b]

Wind parameters		Gene flow frequency	Distance from the pollen source [c]								
			0.5	1	2	4	10	15	25	35	50
			m								
Wind speed	*r* (Pearson)	0.16*	0.12*	0.28**	0.18*	0.31**	0.42**	0.28*	0.14*	0.06	0.01
	df	267	29	21	33	37	29	35	30	25	25
Wind frequency	*r* (Pearson)	0.21*	0.15*	0.12**	0.19*	0.11*	0.39**	0.01	0.05	0.04	-0.05
	df	267	29	21	33	37	29	35	30	25	25
Wind run	*r* (Pearson)	0.19*	0.13*	0.18**	0.12*	0.23*	0.38**	0.09	0.10*	0.03	0.01
	df	267	29	21	33	37	29	35	30	25	25

[a] Pearson correlation coefficients were tested at two significance levels, $P < 0.05$ (*), and $P < 0.01$ (**).
[b] Abbreviations: *df*, degrees of freedom.
[c] Gene flow at 50 m was from the four ordinal directions (NE, NW, SE, SW) in 2014 and 2015.

Table 4.3. Pearson correlation coefficients (*r*) between wind parameters (wind speed, wind frequency, and wind run) and frequency of gene flow at different distances [a, b].

Wind parameters		Gene flow frequency	Distance from the pollen source [c]									
			0.1	0.5	1	2	4	10	15	25	35	50
			m									
Wind speed	*r* (Pearson)	0.30**	0.34**	0.13*	0.18*	0.17*	0.14*	0.19*	0.28**	0.14*	0.06	0.02
	df	403	38	49	35	39	39	25	35	30	25	14
Wind frequency	*r* (Pearson)	0.15**	0.41**	0.19*	0.12*	0.14*	0.22*	0.21*	0.22*	0.08	0.03	- 0.05
	df	403	38	49	35	39	39	25	35	30	25	14
Wind run	*r* (Pearson)	0.16**	0.26**	0.18*	0.15*	0.16*	0.19*	0.13*	0.10*	0.06	0.08	- 0.01
	df	403	38	49	35	39	39	25	35	30	25	14

[a] Pearson correlation coefficients were tested at two significance levels, $P < 0.05$ (*), and $P < 0.01$ (**).
[b] Abbreviations: *df*, degrees of freedom.
[c] Gene flow at 50 m was from the four ordinal directions (NE, NW, SE, SW) in 2014 and 2015.

Table 4.4. Pollen-mediated gene flow from glyphosate-resistant to –susceptible giant ragweed in a field experiment conducted at Clay Center, NE in 2014.

Distance from pollen-source	Plants screened[a]	Plants with glyphosate resistance trait	Frequency of gene flow[b]	Power, $(1-\beta)$[c], $\alpha=0.05$
—— m ——	——— # ———	——— # ———		
0.5	2,520	1,713	0.68	> 0.95
1	2,037	1,222	0.60	> 0.95
2	2,958	1,153	0.39	> 0.95
4	2,456	957	0.39	> 0.95
10	2,325	511	0.22	> 0.95
15	2,165	281	0.13	0.88
25	2,886	346	0.12	0.95
35	5,370	376	0.07	0.95
50	23,820	730	0.03	0.90
Total	46,537	7,289		

[a] Total number of giant ragweed plants screened from all the eight directions at a specific distance from the pollen source.

[b] Average pollen-mediated gene flow frequency from all the eight directions. Frequency of gene flow was determined by using Equation, *Frequency of gene flow* $=\frac{Number\ of\ surviving\ plants}{Number\ of\ sprayed\ plants}$.

[c] Value of power was calculated from a 95% confidence interval using the procedure described by Jhala et al. (2011).

Table 4.5. Pollen-mediated gene flow from glyphosate-resistant to –susceptible giant ragweed in a field experiment conducted at Clay Center, NE in 2015.

Distance from pollen-source	Plants screened[a]	Plants with glyphosate resistance trait	Pollen-mediated gene flow[b]	Power, $(1-\beta)^c$, $\alpha=0.05$
—— m ——	——# ——	————— # —————		
0.1	5,198	2,218	0.43	> 0.95
0.5	5,941	1,645	0.28	> 0.95
1	5,247	1,120	0.21	> 0.95
2	6,696	1,535	0.23	> 0.95
4	6,376	1,536	0.24	> 0.95
10	2,003	329	0.16	> 0.95
15	4,782	632	0.13	0.88
25	3,661	376	0.10	0.95
35	3,221	231	0.07	0.80
50	9,305	456	0.05	0.92
Total	52,430	10,078		

[a] Total number of giant ragweed plants screened from all the eight directions at a specific distance from the pollen source.

[b] Average pollen-mediated gene flow percentage from all the eight directions. Frequency of gene flow was determined by using Equation, *Frequency of gene flow* $=\frac{Number\ of\ surviving\ plants}{Number\ of\ sprayed\ plants}$.

[c] Value of power was calculated from a 95% confidence interval using using the procedure described by Jhala et al. (2011).

Table 4.6. Estimates of the distances where the frequency of gene flow reduced by 50% (O_{50}) and 90% (O_{90}) in 2014 and 2015 and their respective confidence intervals from logistic regression analysis[a].

Direction	2014				2015			
	O_{50}	*CI*	O_{90}	*CI*	O_{50}	*CI*	O_{90}	*CI*
	m							
N	1.3	0.4;4.1	45.6	32.1;64.4	0.4	0.3;0.5	106.5	79.3;142.2
S	2.8	0.8;4.3	28.6	20.5;40.1	0.4	0.3;0.4	37.1	27.3;49.6
E	4.5	3.4;5.4	27	22.7;32.2	1.1	0.8;1.5	19	16.6;21.6
W	7.0	5.8;8.3	49.5	42.7;57.5	2.4	2;2.8	26.4	24;29.1
NE	1.3	0.5;2.6	35.1	25.4;48.3	0.5	0.4;0.6	4.4	3.6;5.4
NW	1.4	1.3;1.6	4.9	4.2;5.6	0.3	0.3;0.4	46.9	34.5;63.5
SE	2.5	0.7;3.9	25.5	20.2;32.3	0.6	0.4;0.7	29.4	24.5;35.3
SW	5.1	4.7;5.4	17.5	16.1;19.0	0.3	0.2;0.3	26	13.6;53.6

[a] O_{50} and O_{90} are the distances where 50% and 90% reduction in gene flow occurred; *CI* is the 95% confidence interval, which includes the lower and upper limits.

Table 4.7. Estimation of the coefficients, standard error, and test of significance for the double-exponential decay model[a] for the prediction of gene flow from glyphosate-resistant giant ragweed under field conditions.

Coefficients[a]	Estimate	Std. Error	z value	P-value[b]
β_0	-3.50	0.09	-39.57	< 2.0e-16***
β_1	0.26	0.10	2.56	0.0103*
γ_1	-5.35	1.06	-5.04	4.74e-07***
β_2	1.59	0.07	22.40	< 2.0e-16***
γ_2	-0.03	0.01	-2.65	0.0081**
β_2:Direction N	-0.25	0.12	-2.19	0.0281*
β_2:Direction NE	-0.59	0.09	-6.10	1.04e-09***
β_2:Direction NW	1.04	0.10	10.20	< 2.0e-16***
β_2:Direction S	0.09	0.11	0.78	0.4343
β_2:Direction SE	-0.12	0.12	-0.98	0.3260
β_2:Direction SW	1.21	0.10	11.52	< 2.0e-16***
β_2:Direction W	-0.17	0.08	-2.18	0.0293*
γ_2:Direction N	-0.02	0.02	-0.69	0.4877
γ_2:Direction NE	0.24	0.04	6.18	6.35e-10***
γ_2:Direction NW	-0.52	0.06	-9.19	< 2.0e-16***
γ_2:Direction S	-0.02	0.02	-0.80	0.4235
γ_2:Direction SE	-0.02	0.02	-1.25	0.2080
γ_2:Direction SW	-0.05	0.03	-1.66	0.0955
γ_2:Direction W	0.02	0.01	2.09	0.0361*
β_2:Year 2	-0.23	0.04	-6.10	1.04e-09***
γ_2:Year 2	-0.02	0.01	-2.41	0.0156*
β_2:Direction N:Year 2	-0.04	0.06	-0.66	0.5074
β_2:Direction NE:Year 2	0.31	0.05	5.45	5.04e-08***
β_2:Direction NW:Year 2	-0.78	0.06	-12.81	< 2.0e-16***
β_2:Direction S:Year 2	-0.23	0.06	-3.66	0.0002***
β_2:Direction SE:Year 2	-0.02	0.06	-0.31	0.7520
β_2:Direction SW:Year 2	-0.94	0.07	-13.12	< 2.0e-16***
β_2:Direction W:Year 2	0.12	0.04	2.89	0.0038**
γ_2:Direction N:Year 2	0.03	0.00	3.32	0.0008***
γ_2:Direction NE:Year 2	-0.23	0.03	-6.29	3.13e-10***
γ_2:Direction NW:Year 2	0.27	0.03	9.44	< 2.0e-16***
γ_2:Direction S:Year 2	0.02	0.01	1.68	0.0912
γ_2:Direction SE:Year 2	0.02	0.01	2.07	0.0383*
γ_2:Direction SW:Year 2	0.01	0.02	0.53	0.5926
γ_2:Direction W:Year 2	-0.01	0.00	-0.49	0.6238

[a] $logit(p_i) = \beta_0 + \exp[\beta_1 + \gamma_1 \times Distance] + \exp[\beta_2(Direction{:}Year) + \gamma_2(Direction{:}Year) \times Distance]$, where p_i is frequency of gene flow of the i^{th} observation; β_0 is the overall intercept; β_1 and β_2 are the intercepts for the first and second instances, respectively; and γ_1, and γ_2 are the decay rates.

[a] β_2 and γ_2 vary with the direction and the year. In this table, β_2 and γ_2 show the intercept and decay rate, respectively, for one direction (East) in year 1 (2014). However, "β_2:Direction", or "β_2:Year 2" denote the

change (from East direction and year 1) in β_2 for other directions and year 2 (2015), respectively. The same is true for γ_2.

[b] P-values show the test of significance at P < 0.05 (*) and P < 0.01 (**).

Table 4.8. AIC values, and AIC differences (Δ) for the possible models to predict pollen-mediated gene flow (PMGF) under field conditions[a].

No.	Model[b]	*K*	*AIC*	Δ*AIC*	*LL*
1	GF~ Exp(1+dist) + Exp(1+dist*direc*yr)	35	8282.84	0	-4106.42
2	Exp(dist*direc*yr)+Exp(dist)	34	8329.424	46.58	-4130.71
3	Exp(dist*direc*yr)+Exp(dist*direc*yr)	33	8528.733	245.89	-4231.37
4	Exp(1+dist*direc*yr)	33	8700.491	417.65	-4317.25
5	Exp(dist)+Exp(dist+direc*yr)	18	9107.713	824.87	-4535.86
6	Exp(dist+direc*yr)+Exp(dist)	19	9404.469	1121.63	-4683.23
7	Exp(dist+direc*yr)+Exp(dist+direc*yr)	18	9465.676	1182.84	-4714.84
8	Exp(dist)+Exp(dist*wrun*yr)	9	10491.89	2209.05	-5236.95
9	Exp(dist*ws*yr)+Exp(dist+wrun)	10	10634.05	2351.21	-5307.03
10	Exp(dist)+Exp(dist*freq*yr)	9	10649.61	2366.77	-5315.81
11	Exp(dist)+Exp(dist*ws*yr)	9	10668.32	2385.48	-5325.16
12	Exp(1+dist)+Exp(dist*ws)	6	10702.53	2419.69	-5345.27
13	Exp(dist)+Exp(dist+wrun*yr)	6	10786.14	2503.3	-5387.07
14	Exp(dist)+Exp(dist*direc)	18	10886.93	2604.09	-5425.47
15	Exp(dist*direc)	17	10919.79	2636.95	-5442.9
16	Exp(dist+ws*yr)+ Exp(dist)	6	11032.06	2749.22	-5510.03
17	Exp(dist*freq*yr)	8	11041.99	2759.15	-5512.99
18	Exp(dist+direc)+Exp(dist+direc)	10	11115.02	2832.18	-5547.51
19	Exp(dist)+Exp(dist+ws*freq)	6	11202.11	2919.27	-5595.05
20	Exp(dist)+Exp(dist*wrun)	5	11202.82	2919.98	-5596.41
21	Exp(dist)+Exp(dist+freq*yr)	6	11248.12	2965.28	-5618.06
22	Exp(dist*wrun)+Exp(dist)	5	11264.35	2981.51	-5627.17
23	Exp(dist+direc)+Exp(dist)	10	11267.32	2984.48	-5623.66
24	Exp(dist)+Exp(dist*ws)	5	11303.57	3020.73	-5646.79
25	Exp(dist)+Exp(dist+direc)	10	11382.92	3100.08	-5681.46
26	Exp(dist*freq*yr)+Exp(dist*freq*yr)	8	11388.55	3105.71	-5686.28
27	Exp(dist*ws)+Exp(dist)	5	11388.61	3105.77	-5689.3
28	Exp(dist)+ Exp(dist+wrun)	4	11635.37	3352.53	-5813.68
29	Exp(dist+ws)+Exp(dist)	4	11689.66	3406.82	-5840.83
30	Exp(dist+freq*yr)+Exp(dist+freq*yr)	5	11743.33	3460.49	-5866.67
31	Exp(dist*ws*yr)+Exp(dist*ws*yr)	8	11819.4	3536.56	-5817.02
32	Exp(dist+freq*yr)	5	11952.81	3669.97	-5971.41
33	Exp(dist*wrun*yr)+Exp(dist*wrun*yr)	8	12096.03	3813.19	-6040.01
34	Exp(dist*freq)+ Exp(dist)	5	12145.59	3862.75	-6067.8
35	Exp(dist+ws)+ Exp(dist+ws)	4	12147.38	3864.54	-6069.69

36	Exp(dist+ws*yr)+Exp(dist+ws*yr)	5	12221.14	3938.3	-6105.57
37	Exp(dist+wrun*yr)+Exp(dist+wrun*yr)	5	12590.2	4307.36	-6290.1
38	Exp(dist*ws)+Exp(dist*ws)	4	12968.52	4685.68	-6480.26
39	Exp(dist*wrun)+ Exp(dist*wrun)	4	13164.19	4881.35	-6578.1
40	Exp(dist+wrun)+ Exp(dist+wrun)	3	13233.39	4950.55	-6613.7
41	Exp(dist*freq)+ Exp(dist*freq)	4	13290.39	5007.55	-6641.2
42	Exp(dist)+Exp(dist)	2	13345.23	5062.39	-6670.62
43	Exp(dist+freq)+Exp(dist+freq)	3	13345.8	5062.96	-6669.9

[a] *AIC* is the Akaike's Information Criterion calculated using $AIC = -2LL + 2K$; K is the number of parameters; LL is the maximized log likelihood.

[b] dist = distance from the pollen source; direc = directions of the pollen receptor blocks; GF = gene flow frequency; PMGF = pollen-mediated gene flow; ws = wind speed; freq = wind frequency; wrun = wind run (i.e., ws × freq); yr = year.

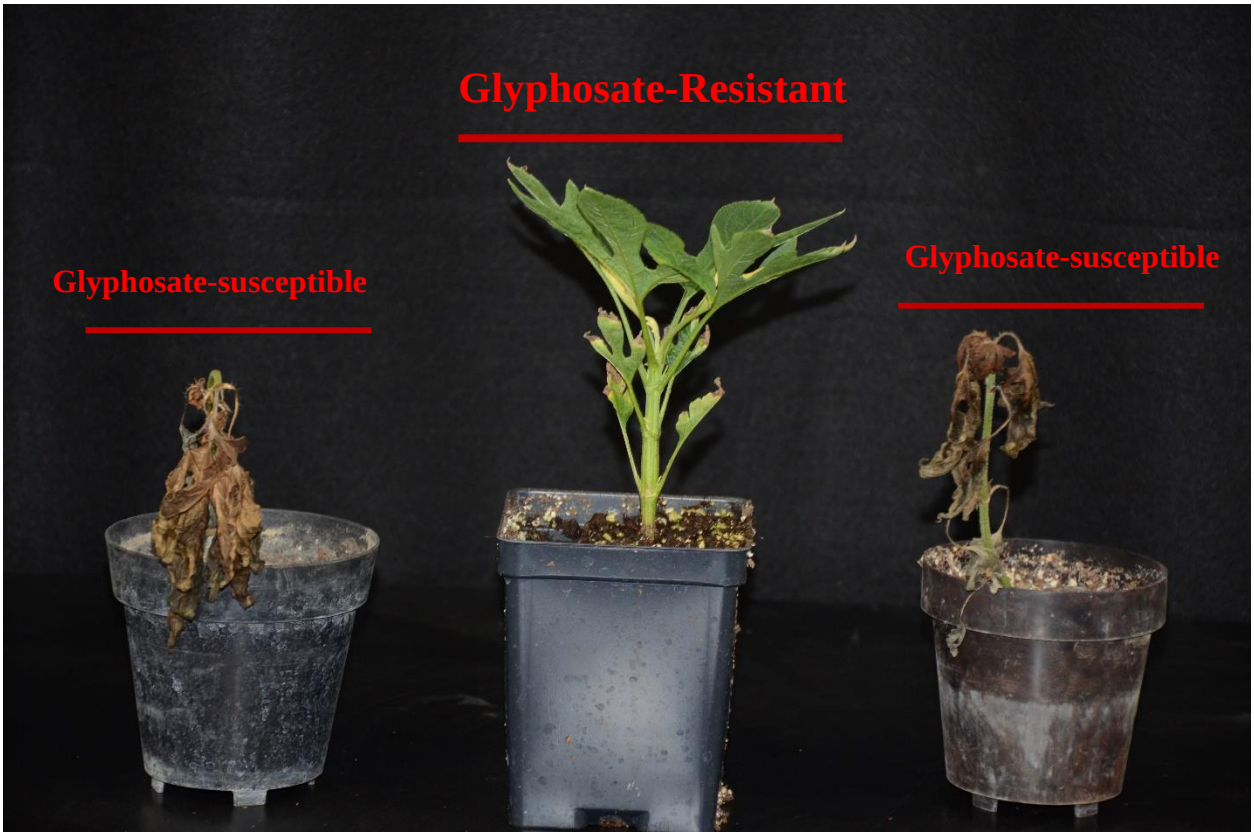

Figure 4.1. Glyphosate-resistant and -susceptible giant ragweed plants at 14 d after treatment with glyphosate at 2× rate (1× = 1,260 g ha^{-1}) from the respective biotypes used as pollen-source and -receptors in gene flow study conducted in Nebraska.

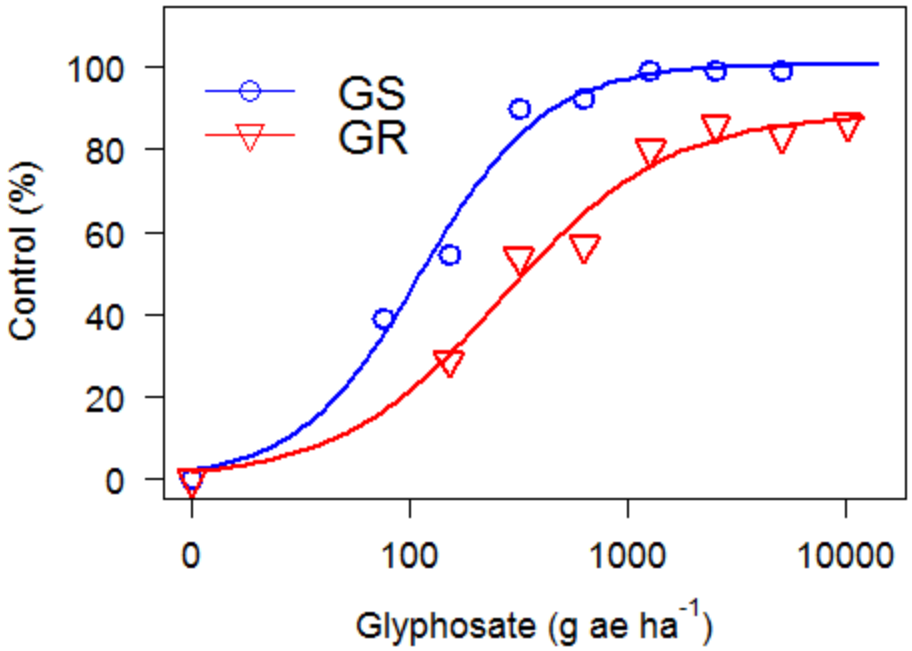

Figure 4.2. Dose-response bioassay of glyphosate-resistant (GR) and –susceptible (GS) giant ragweed used as parent biotypes in this study. The visual control rating was taken at 21 d after glyphosate application.

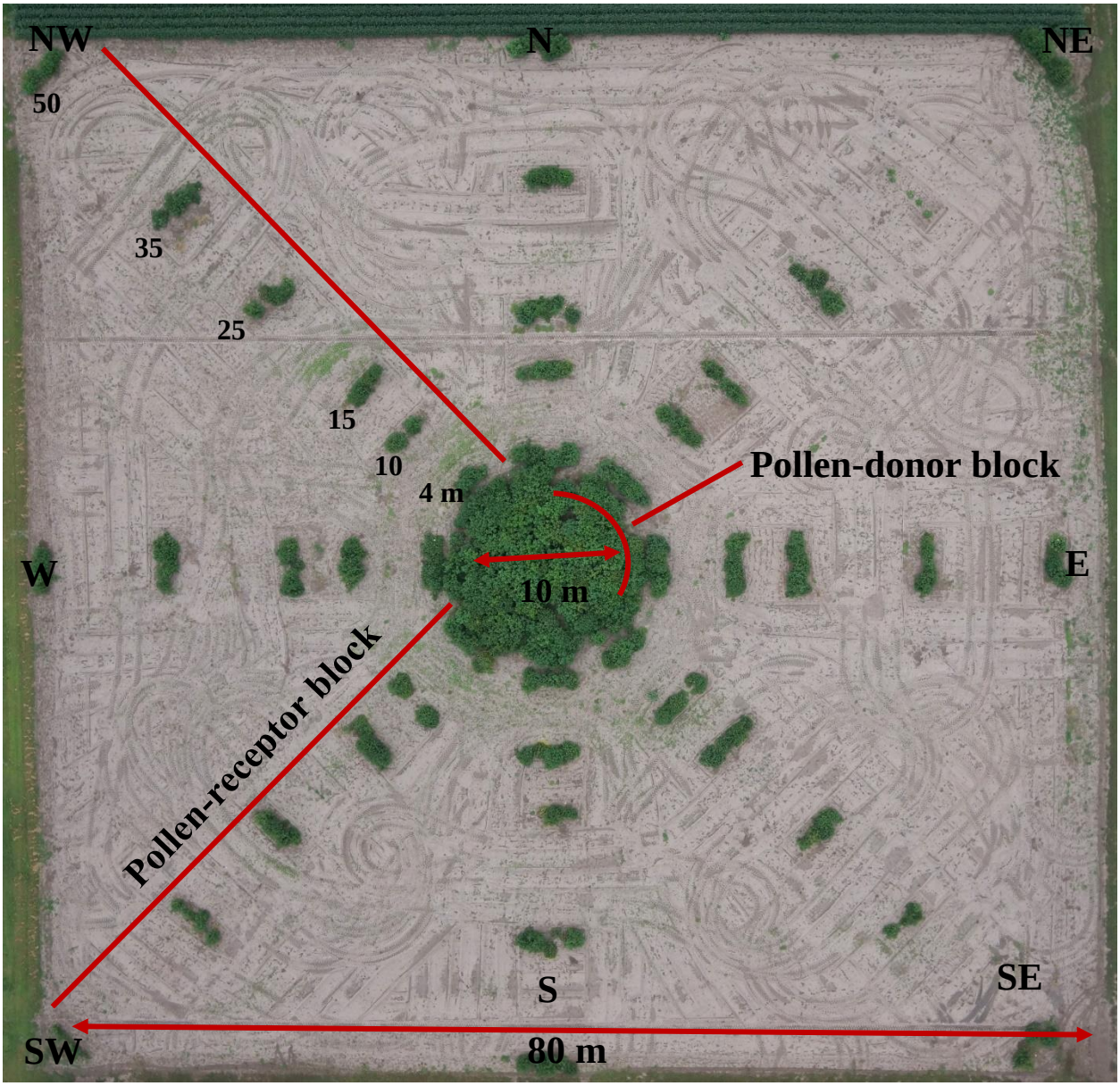

Figure 4.3. Aerial view of the field experiment conducted to quantify pollen-mediated gene flow from glyphosate-resistant to –susceptible giant ragweed at South Central Agricultural Laboratory (SCAL), Clay Center, NE. Glyphosate-resistant giant ragweed plants were transplanted in the pollen-donor block of 10 m diam in the center of the field. The surrounding pollen-receptor area (80 m × 80 m) was divided into eight directional blocks where glyphosate-susceptible giant ragweed plants were transplanted. Giant ragweed seeds were harvested at maturity from specific distances along the eight directional arms.

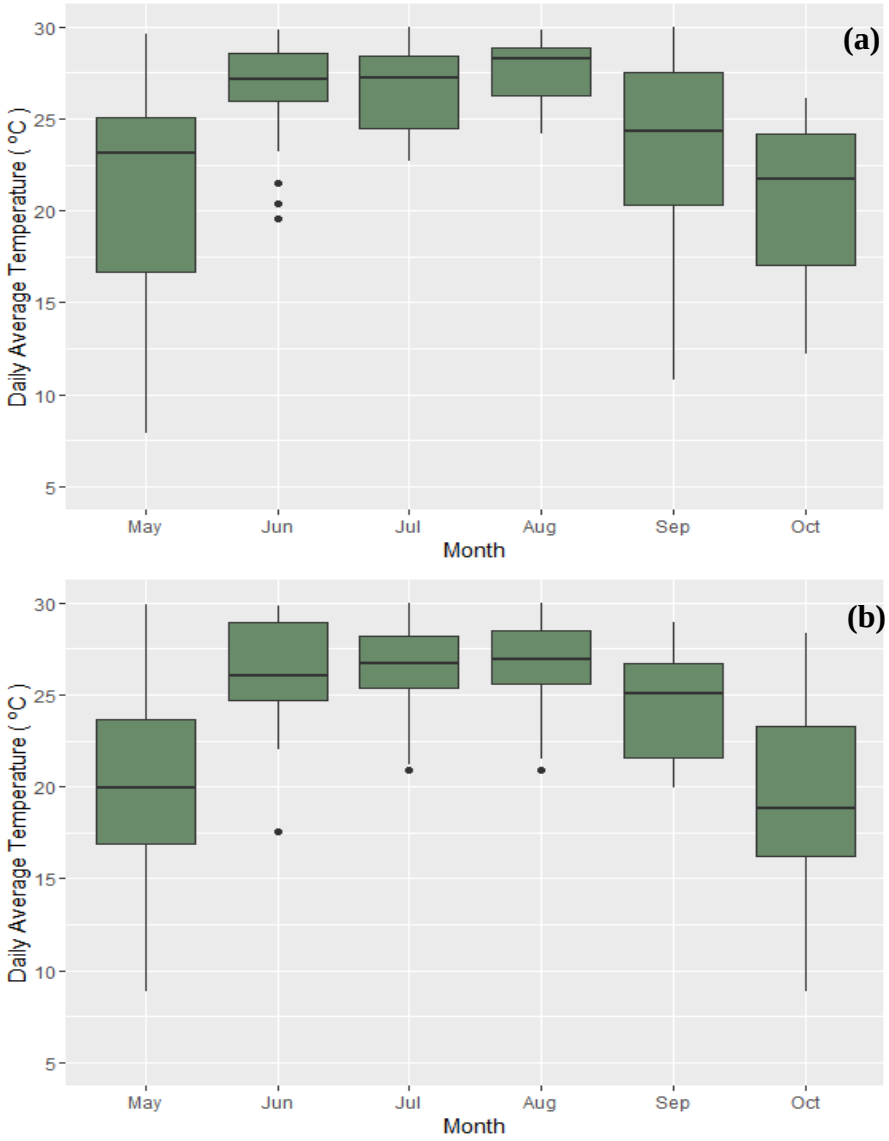

Figure 4.4. Daily average air temperature (C) from May to October in (a) 2014 and (b) 2015 at the South Central Agricultural Laboratory (SCAL), Clay Center, NE. The boxplots show the variation in daily average temperature (^{0}C) for each month during which field studies were conducted in 2014 and 2015.

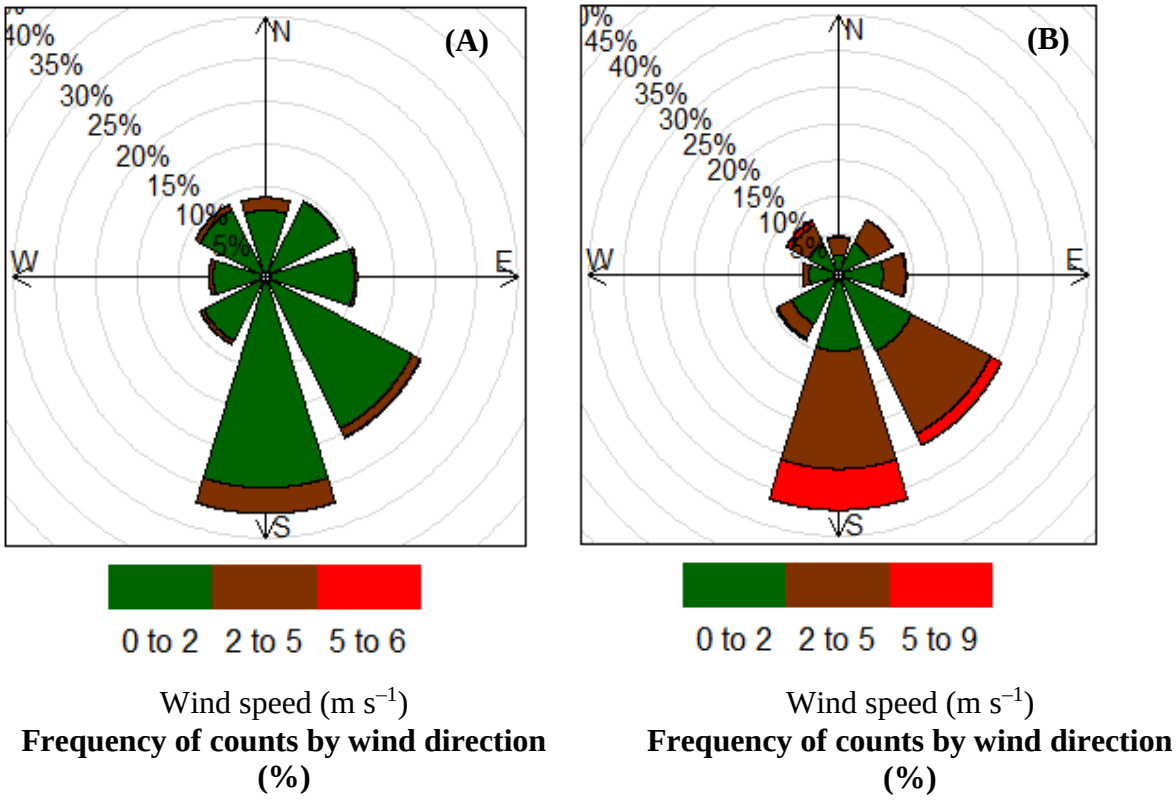

Figure 4.5. Wind rose plots displaying wind speed (m s^{-1}) and wind frequency (%) in four cardinal (N, S, E, W) and four ordinal (NE, NW, SE, SW) directions during the flowering period for giant ragweed in (A) 2014 and (B) 2015 at the experimental site at South Central Agricultural Laboratory (SCAL), Clay Center, NE. The plots show the direction from which the wind was blowing in particular year.

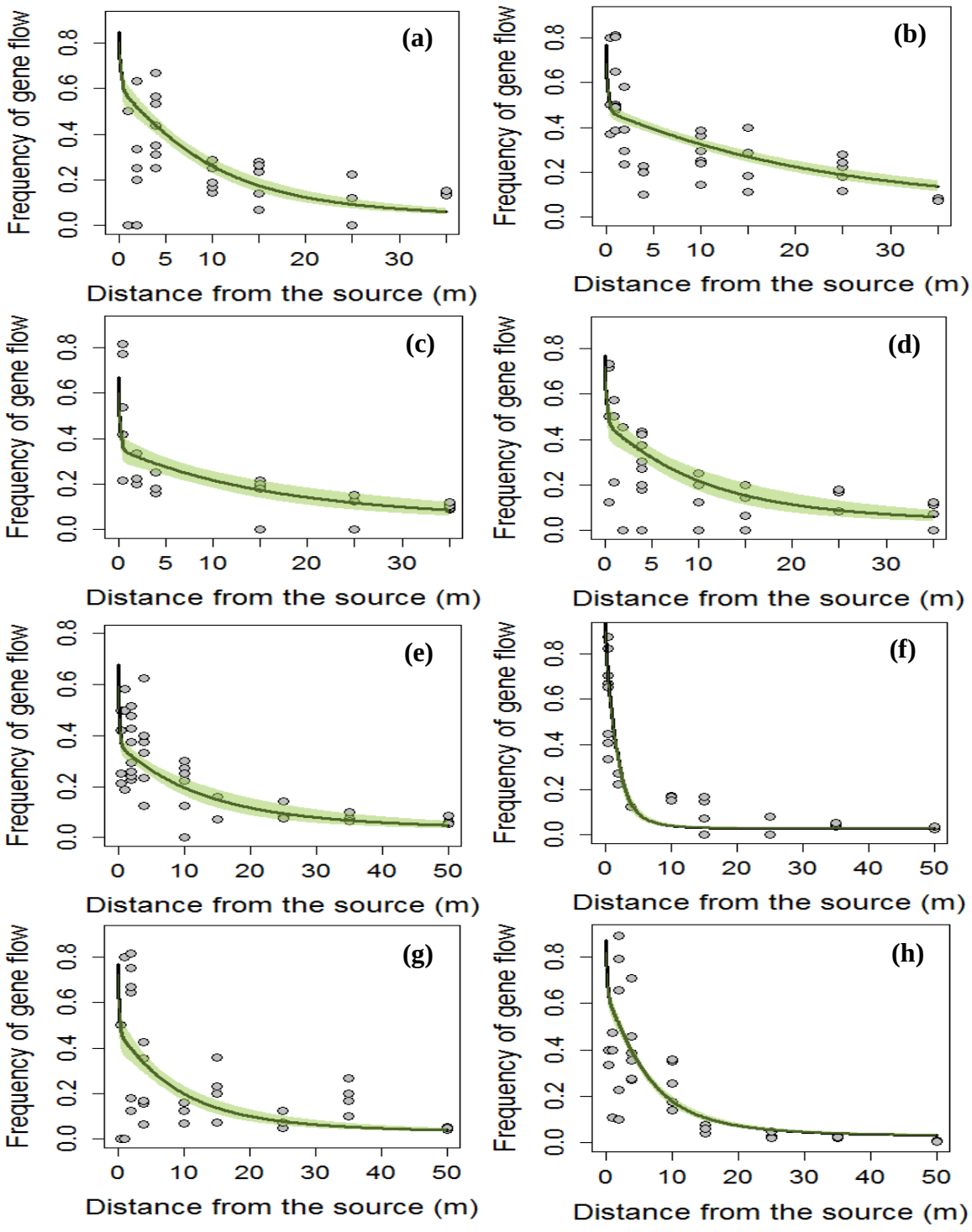

Figure 4.6. Pollen-mediated gene flow from glyphosate-resistant to –susceptible giant ragweed affected by distance (m) from the pollen source in eight directions: (a) East, (b) West, (c) North, (d) South, (e) Northeast, (f) Northwest, (g) Southeast, (h) Southwest in 2014. The green shaded area represents the 95% confidence intervals for prediction plots.

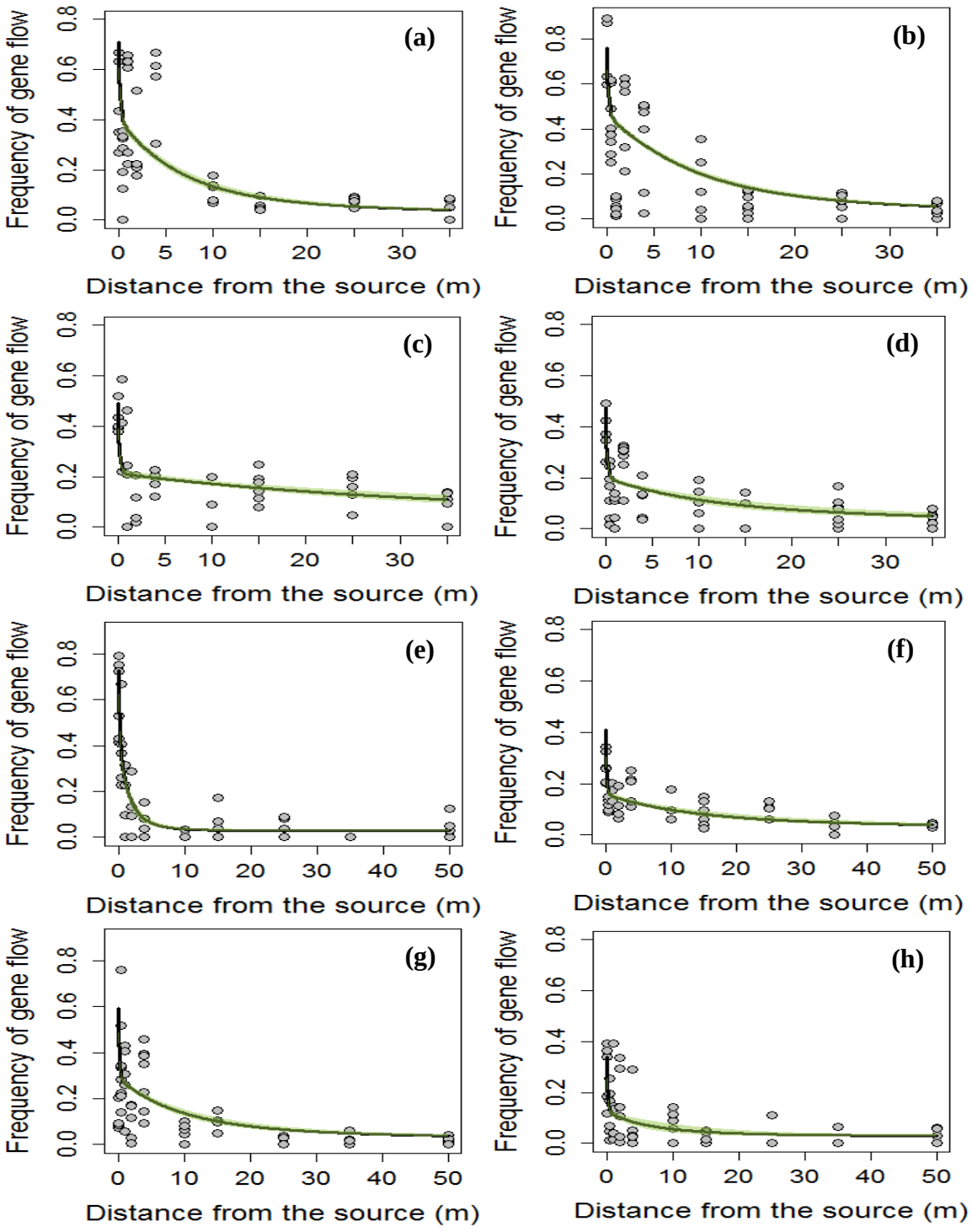

Figure 4.7. Pollen-mediated gene flow from glyphosate-resistant to –susceptible giant ragweed affected by distance (m) from the pollen source in eight directions: (a) East, (b) West, (c) North, (d) South, (e) Northeast, (f) Northwest, (g) Southeast, (h) Southwest in 2015. The green shaded area represents the 95% confidence intervals for prediction plots

CHAPTER 5: INTEGRATED MANAGEMENT OF GLYPHOSATE-RESISTANT GIANT RAGWEED (*Ambrosia trifida*) WITH TILLAGE AND HERBICIDES IN SOYBEAN

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Abstract

Giant ragweed is one of the most competitive annual broadleaf weeds in soybean production fields in the Midwestern United States and eastern Canada due to its early emergence, rapid growth rate, high plasticity, and resistance to glyphosate and acetolactate synthase (ALS) inhibitors. Therefore, early season management of giant ragweed is critical to avoid yield loss. The objectives of this study were to evaluate control of glyphosate-resistant giant ragweed through the integration of preplant tillage or 2,4-D; PRE or early POST (EPOST) followed by (fb) late POST (LPOST) herbicide programs with or without preplant tillage or 2,4-D, and their effect on soybean injury and yield. A field study was conducted in 2013 and 2014 in David City, NE in a field infested with glyphosate-resistant giant ragweed. Preplant tillage or 2,4-D application provided > 90% control of glyphosate-resistant giant ragweed 14 d after preplant treatment (DAPT). Giant ragweed control and biomass reduction was consistently > 90% with preplant tillage or 2,4-D fb sulfentrazone plus cloransulam PRE or glyphosate plus cloransulam EPOST fb glyphosate plus fomesafen or lactofen LPOST compared to ≤ 86% control with same treatments without preplant tillage or 2,4-D. PRE or EPOST fb LPOST herbicide programs preceded by preplant treatments resulted in giant ragweed density < 2

plants m^{-2} and soybean yield > 2,400 kg ha^{-1} compared to the density of ≥ 2 plants m^{-2} and soybean yield < 1,800 kg ha^{-1} under PRE or EPOST fb LPOST herbicide programs. The contrast analysis also indicated preplant tillage or 2,4-D fb PRE or POST program was more effective for giant ragweed management compared to PRE fb POST herbicide programs. Integration of preplant tillage would provide an alternate method for early season control of giant ragweed; however, a follow up application of herbicides are needed for season-long control in soybean.

Introduction

Giant ragweed, a member of the Asteraceae family, is a highly competitive summer annual broadleaf weed. Giant ragweed is native to the United States and known for its allergenic pollen grains that are a major cause of hay fever (Kil et al. 2004; Rybnicek and Jager 2001). Historically, giant ragweed was commonly found in non-crop areas, including stream banks, flood plains, right-of-way, fence lines, and disturbed locations (Abdul-Fatih and Bazzaz 1979; Bassett and Crompton 1982). However, over the last two decades, giant ragweed has adapted to agricultural cropping systems and become a challenging weed in several agronomic crops (Johnson et al. 2006; Norsworthy et al. 2010; Steckel 2007; Vink et al. 2012a). Due to early emergence, rapid growth rate, large leaf size, high photosynthetic rate, and ability to germinate and survive in diverse environments (Abdul-Fatih and Bazzaz 1979; Bazzaz and Carlson 1979; Harrison et al. 2001), giant ragweed has a competitive advantage in agronomic crops early in the season compared to other weed species, such as grasses, that emerges relatively late (Werle et al. 2014). In addition, the evolution of wider window of emergence over the years particularly in arable fields, high plasticity in plant vigor, and rapid biomass

accumulation allows giant ragweed to dominate over all other vegetation in its vicinity (Davis et al. 2013; Glettner and Stoltenberg 2015; Kelly et al. 2012; Schutte et al. 2008; 2012).

Giant ragweed is a major weed in corn (*Zea mays* L.), soybean, and cotton (*Gossypium hirsutum* L.) and is enumerated as one of the most problematic and economically important weeds in Illinois, Indiana, Kentucky, Minnesota, Nebraska, Ohio, and Oklahoma (Johnson et al. 2004; Jordan 1985; Loux and Berry 1991). Previous studies have evaluated the competition of giant ragweed in corn, soybean, and cotton, and indicated that giant ragweed is most competitive in soybean even at low densities (Barnett and Steckel 2013; Baysinger and Sims 1991; Harrison et al. 2001). For instance, a yield reduction of 45 to 50% has been documented with 2 giant ragweed plants 9-m^{-1} of row length in soybean (Baysinger and Sims 1991). Webster et al. (1994) reported up to 77% reduction in soybean yield with interference of 1 giant ragweed plant m^{-2}. Additionally, Webster et al. (1994) documented two different growth habits employed by giant ragweed to take competitive advantage over soybean at low densities. Early in the season, giant ragweed emerges rapidly and outgrows the crop in height to create a shading effect with little growth within the canopy. However, late in the season when its primary leaves begin to abscise, especially after the closure of the crop canopy, axillary leaves are produced within the canopy. These late emerging axillary leaves are more shade tolerant, allowing giant ragweed to compete for light and resources not only above, but also within the soybean canopy (Regnier and Stoller 1989; Webster et al. 1994).

The critical period of weed control in soybean is 4 to 6-wk after planting (Bloomberg et al. 1982; Coble et al. 1981; Williams and Hayes 1984); however, to avoid

soybean yield losses due to giant ragweed interference, its critical period extends from 8 to 10-wk after soybean emergence (WAE) (Baysinger and Sims 1991). Harrison et al. (2001) reported 76 to 87% reduction in yield losses with a 4-wk delay in emergence of giant ragweed in corn compared to losses with concurrent emergence. Therefore, early season control of giant ragweed is essential to reduce yield losses and can provide the crops with an initial competitive advantage. Historically, acetolactate synthase (ALS) inhibitors such as cloransulam-methyl, chlorimuron-ethyl, and imazethapyr were used for giant ragweed control (Franey and Hart 1999). However, giant ragweed control options were reduced within a short-frame of time when ALS inhibitor-resistant biotypes were reported in several states including Indiana, Illinois, Iowa, and Ohio (Heap 2015; Patzoldt and Tranel 2002; Taylor et al. 2002; Zelaya and Owen 2004).

The commercialization and rapid adoption of glyphosate-tolerant soybean after 1997 enabled producers to effectively control giant ragweed including ALS inhibitor-resistant biotypes with glyphosate (Stachler 2008). However, the repeated and continuous use of glyphosate in glyphosate-tolerant corn and soybean resulted in the evolution of glyphosate-resistant giant ragweed. It was first confirmed in 2004 in Ohio and subsequently in 11 states including Arkansas, Indiana, Iowa, Kansas, Kentucky, Minnesota, Mississippi, Missouri, Nebraska, Tennessee, and Wisconsin (Heap 2015); and in Ontario, Canada (Sikkema et al. 2009; Vink et al. 2012a). The potential causes for the large-scale prevalence of glyphosate-resistant giant ragweed are the continuous use of glyphosate over several years, limited or no use of PRE herbicides, and shift towards no-till cropping systems (Ferrell and Witt 2002; Givens et al. 2009; Powles and Yu 2010; Young 2006). Moreover, since no herbicides with new modes of action have been

introduced to the market for over two decades (Green 2014), the POST herbicide options for control of herbicide-resistant weeds, including giant ragweed, are limited (Duke 2012). Therefore, diversification of weed management programs is urgently needed that should include non-chemical options such as cover crops, tillage, crop rotation, and harvest and destruction of weed seeds to reduce weed seedbank addition (Shaner and Beckie 2014; Norsworthy et al. 2012; Walsh et al. 2013).

Historically, tillage has been one of the most important methods for weed control in agricultural crops (Shrestha et al. 2006). Tillage usually affects weeds by splitting shoots from roots, uprooting, or covering unwanted vegetation, by stirring weed seeds both vertically and horizontally and modifying the soil environment to promote or inhibit seed germination and establishment (Clements et al. 1996; Shaw et al. 2012; Swanton et al. 2000). Wilson (1993) reported 86% reduction in weed density with preplant tillage compared to nontreated control, and observed broad-spectrum weed control by integrating preplant tillage with herbicides compared to tillage or herbicides alone. In addition, tillage integrated with herbicides has been substantial for the management of important herbicide-resistant weeds, such as Palmer amaranth (*Amaranthus palmeri* S. Wats.) in the southern United States (Aulakh et al. 2012; Culpepper et al. 2009; Kelton et al. 2013).

Currently, protoporphyrinogen oxidase (PPO)-inhibitors and some ALS-inhibiting herbicides, particularly cloransulam-methyl, are frequently used for control of giant ragweed in soybean (Knezevic 2015; Vink et al. 2012b). Several studies have reported effective (>89%) control of giant ragweed with PPO-inhibitors such as bentazon, carfentrazone, flumioxazin, and fomesafen (Norsworthy et al. 2010; 2011). However,

dependence on herbicide(s) with the same mode of action for control of troublesome weeds, such as giant ragweed, has a potential risk for evolution of new herbicide-resistance. In addition, for early and late season control of glyphosate-resistant giant ragweed, diverse strategies are needed that will allow the planting of soybean in a weed-free environment and prevent the enrichment of the weed seedbank in the soil (Bagavathiannan and Norsworthy 2012; Norsworthy et al. 2012). Scientific literature is not available on the effect of early spring tillage on the control of giant ragweed.

The objectives of this study were to evaluate an integrated approach for the management of glyphosate-resistant giant ragweed in glyphosate-tolerant soybean by determining: 1) the effectiveness of preplant tillage or 2,4-D, and 2) the relative effectiveness of PRE fb POST vs EPOST fb LPOST herbicide programs with or without preplant tillage or 2,4-D and their impact on soybean injury and yield. We hypothesized that preplant tillage or 2,4-D fb PRE or EPOST fb LPOST herbicides would result in early and late-season control of glyphosate-resistant giant ragweed compared to PRE or EPOST fb LPOST herbicide programs.

Materials and Methods

A field study was conducted at David City (41.25°N, 97.13°W), NE in 2013 and 2014 in a grower's field infested with glyphosate-resistant giant ragweed. A giant ragweed biotype from this site was confirmed to be resistant to glyphosate in 2011 with the level of resistance ranging from 14 to 36x [where x is the labeled rate of glyphosate (i.e, 1,260 g ae ha^{-1}) required for > 90% control of susceptible populations] compared to susceptible biotypes (Rana et al. 2013). The level of resistance was determined by calculating a ratio of glyphosate rate required for 90% control (ED_{90} value) of

glyphosate-resistant and susceptible giant ragweed biotypes. The density of glyphosate-resistant giant ragweed at this site was 18 to 30 plants m^{-2}. The soil texture of the experimental site was silty loam with a pH of 5.4, and a composition of 18% sand, 50% silt, 32% clay, and 2.1% organic matter (AgSource Laboratories-Lincoln, NE 68502). Glyphosate-resistant soybean seeds [Cv. 'Pioneer 93Y12' (2013) and 'NK S28U7' (2014)] were planted 3 cm deep on May 24, 2013 and May 17, 2014. Individual plots were 3 m wide and 9 m long, containing four soybean rows spaced 76 cm apart. The treatments were arranged in a split-plot design with four replications, where the main plot was preplant control methods (preplant tillage, 2,4-D or no preplant control), and the sub-plot was PRE/POST herbicide treatments. A total of 12 treatment combinations, including preplant tillage or 2,4-D application, or no preplant control followed by (fb) PRE and/or POST herbicides were compared for control of glyphosate-resistant giant ragweed in soybean (Table 5.1). A treatment with no preplant tillage and/or herbicide application served as a nontreated control for comparison. The application rates of herbicides were selected based on the labeled rates in soybean.

Preplant tillage was accomplished using a tandem disk harrow on May 10, 2013 and May 3, 2014 and 2,4-D was applied on the same day during both years. Herbicide treatments were applied as PRE (May 24, 2013 and May 17, 2014), early POST (EPOST) (June 14, 2013 and June 10, 2014), and late POST (LPOST) (June 28, 2013 and June 30, 2014). Herbicides were applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at 276 kPa equipped with a four-nozzle boom fitted with AIXR 110015 flat-fan nozzles (TeeJet, Spraying Systems Co., P. O. Box 7900, Wheaton, IL

60189). The experimental site was under rain-fed/dryland conditions during both years without any supplemental irrigation.

During both years, data were collected for visual control estimates of giant ragweed using a scale of 0 (no control) to 100% (complete control) at 7 and 14 d after preplant treatments (DAPT); 7, 14, and 21 d after PRE (DAPRE) herbicide treatments; 30 and 60 d after early POST (DAEPOST) herbicide treatments, and at harvest. Herbicide injury symptoms on soybean (if any) were recorded using a scale of 0 (no injury) to 100% (plant death) at 7, 14, and 21 d after herbicide treatments. Glyphosate-resistant giant ragweed density was recorded from two randomly selected 0.25-m^2 quadrats per plot at 30 and 60 d after EPOST herbicide treatments and two wk before soybean harvest. Glyphosate-resistant giant ragweed biomass was assessed from the same two 0.25-m^2 quadrats per plot randomly selected for density data at 60 d after EPOST. Giant ragweed plants that survived herbicide treatments were cut at the stem base close to the soil surface, placed in paper bags, dried in an oven for 72 h at 50 C, and the dried biomass was weighed (g). Soybeans were harvested using a plot combine and yields were adjusted to 13% moisture content. Giant ragweed biomass data were converted into percent shoot biomass reduction compared to the nontreated control (Wortman 2014) as:

$$\text{Percent shoot biomass reduction} = [(\bar{C} - B)/\bar{C}]^* 100 \quad [1]$$

where, $\bar{C}$ is the mean biomass of the four nontreated control replicates, and B is the biomass of an individual treated experimental unit.

Statistical Analysis. Data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc, Cary, NC). Data of visual control estimates of giant ragweed at 7 and 14 DAPT (Figure 5.1) were analyzed as randomized

complete block design with preplant control methods (preplant tillage, 2,4-D, no-preplant control) considered as fixed effect and replication as a random effect in the model. This is because the sub-plot treatments (PRE/POST herbicides) were not applied at this time. The analysis of all other data were performed in split-plot design with year, preplant control methods, herbicide treatments and their interactions considered as the fixed effects, while replication as a random effect in the model. The treatments with zero response variables were not included in the data analysis. Before analysis, data were tested for normality of residuals using the PROC UNIVARIATE procedure. Visual estimates of giant ragweed control, density, and biomass data were arcsine square-root transformed before analysis; however, back-transformed data are presented with mean separation based on the transformed data. If the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ using Tukey-Kramer's pairwise comparison test. A single degree of freedom contrast statements were used to compare herbicide programs with and without preplant treatment, and to compare herbicide programs with different application timings including PRE fb LPOST versus EPOST fb LPOST. Year-by-treatment interaction was not significant; therefore, data of both years were combined for variables including giant ragweed control estimates, density, and biomass.

Results and Discussion

The interaction between main plot treatments (preplant tillage, 2,4-D, no-preplant control) and sub-plot treatments (PRE/POST herbicides) was significant ($P < 0.05$) for all variables including giant ragweed control estimates, density, and biomass. Preplant tillage or 2,4-D application provided 96 and 69% control of glyphosate-resistant giant ragweed, respectively, at 7 DAPT. Giant ragweed control improved to 94% at 14 d after

2,4-D preplant application and was comparable with tillage (Figure 5.1). The improvement is because of systemic activity of 2,4-D and it takes about 10 to 20 d to fully express growth inhibition symptoms on broadleaf weeds (Kelley et al. 2005; Robinson et al. 2013). Jhala et al. (2014) reported ≤ 66% control of glyphosate-resistant giant ragweed 7 d after 2,4-D applied preplant, which improved to > 85% at 14 d after treatment. The application of sulfentrazone plus cloransulam PRE without preplant tillage or 2,4-D resulted in < 75 and 84% control, respectively, at 7 and 21 DAPRE compared to > 96% control when preceded with preplant treatments.

The contrast analysis suggested > 95% control with preplant fb PRE programs compared to PRE-only treatments (< 85%) at 7 and 21 DAPRE (Table 5.2). Similarly, Kaur et al. (2014) reported 68% control of giant ragweed with sulfentrazone plus cloransulam at 7 DAPT. Ganie et al. (2015) reported ≥ 80% control of glyphosate-resistant giant ragweed with preplant tillage at 10 DAPT in corn. Thus, results of this study emphasize the importance of preplant tillage or 2,4-D application for effective management of glyphosate-resistant giant ragweed in soybean because it resulted in ≥ 89% control regardless of PRE herbicide treatments at 7 and 21 DAPRE. Additionally, these results provided further evidence to the recommendations including preplant tillage or herbicide application made by Johnson et al. (2006) for control of emerged giant ragweed plants.

Preplant tillage or 2,4-D resulted in < 71 and < 45% control of giant ragweed at 30 and 60 DAEPOST, respectively (Table 5.2). This was primarily due to the regrowth of partially controlled plants or the new emergence of giant ragweed seedlings after tillage or 2,4-D applied preplant. Similarly, Jhala et al. (2014) reported ≤ 68% control of

glyphosate-resistant giant ragweed at 30 DAPT in soybean when preplant herbicide treatments were not followed by PRE or POST herbicide treatments. Preplant tillage or 2,4-D fb sulfentrazone plus cloransulam PRE fb glyphosate or glyphosate plus fomesafen LPOST or glyphosate plus cloransulam EPOST fb glyphosate plus lactofen LPOST resulted in ≥ 98% control of glyphosate-resistant giant ragweed at 30 and 60 DAEPOST. However, without preplant treatments, sulfentrazone plus cloransulam PRE fb glyphosate or glyphosate plus fomesafen LPOST resulted in 84 to 86% control at 30 DAEPOST, and decreased to ≤ 78% control at harvest (Table 5.2). A similar trend was observed at the harvest. Control of giant ragweed with preplant treatments alone reduced to < 20% (Table 5.2). Results indicated > 95% control of glyphosate-resistant giant ragweed throughout the season is possible with preplant tillage or 2,4-D fb PRE or EPOST fb LPOST herbicide programs (Table 5.2). Similarly, previous research has reported that without effective preplant management, in-crop application of glyphosate tank-mixture with fomesafen/bentazon/chlorimuron-ethyl, or other POST-only herbicide programs provided unacceptable control of glyphosate-resistant giant ragweed (Follings et al. 2013; Riley and Bradley 2012; 2014). However, Vink et al. (2012b) reported that sequential applications of glyphosate plus dicamba applied preplant fb POST resulted in 100% control of glyphosate-resistant giant ragweed in dicamba-tolerant soybean. Moreover, the contrast statements confirmed preplant fb PRE fb LPOST program provided > 95% giant ragweed control compared to < 87% control with PRE fb LPOST program alone, and indicated similar control with PRE fb LPOST and EPOST fb LPOST programs when preceded by preplant treatments (Table 5.2).

The density and percent shoot biomass reduction of glyphosate-resistant giant ragweed reflected the results of visual control estimates. The highest density of giant ragweed (19 to 22 plants m^{-2}) was recorded in the nontreated control plots compared with other treatments (Table 5.3). Preplant tillage or 2,4-D fb PRE or EPOST fb LPOST treatments resulted in a density of < 2 plants m^{-2} and provided season-long control of giant ragweed (Table 5.3). Similarly, Kelton et al. (2013) reported a reduction of Palmer amaranth density to ≤ 4 plants m^{-2} with spring tillage compared to ≥ 4 plants m^{-2} without tillage in cotton. Sulfentrazone plus cloransulam PRE fb glyphosate or glyphosate plus fomesafen LPOST resulted in ≤ 5 plants m^{-2} at 60 DAEPOST but was comparable with preplant tillage or 2,4-D fb PRE or EPOST fb LPOST programs at harvest (Table 5.3). Jhala et al. (2014) reported ≤ 1 giant ragweed plant m^{-2} with 2,4-D preplant fb PRE treatments.

Giant ragweed shoot biomass reduction with preplant-only treatments was < 55%. However, preplant treatments fb PRE or EPOST fb LPOST herbicides resulted in ≥ 95% shoot biomass reduction compared to ≤ 89% reduction with PRE fb LPOST treatments. In comparable studies, 75 to 100% giant ragweed shoot biomass reduction was observed with 2,4-D or saflufenacil preplant fb POST application of glufosinate or ALS plus PPO-inhibiting herbicides (Jhala et al. 2014; Kaur et al. 2014). Similarly, Vink et al. (2012b) reported ≥ 99% reduction in giant ragweed shoot biomass with application of glyphosate plus dicamba preplant fb glyphosate plus dicamba POST in dicamba-tolerant soybean. The contrast analysis indicated low giant ragweed density and high shoot biomass reduction with preplant fb PRE fb LPOST programs compared to PRE fb LPOST programs at 60 DAEPOST. Similarly, PRE fb LPOST programs resulted in lower giant

ragweed density (< 5 plants m^{-1}) and > 85% biomass reduction compared to EPOST fb LPOST programs, irrespective of preplant treatments (Table 5.3).

Soybean injury was 12 to 16% at 14 d after LPOST application of fomesafen or lactofen; however, injuries were transient and had no impact on soybean yield (Table 5.3). Year-by-treatment interaction for soybean yield was significant probably due to differences in rainfall received during 2013 and 2014 (data not shown); hence, soybean yields are presented separately by year (Table 5.3). The nontreated control resulted in no soybean yield due to high giant ragweed density (19 to 22 plants m^{-2}). Similarly, recent studies in Nebraska have reported 100% soybean yield loss when giant ragweed plants (> 15 plants m^{-2}) were allowed to compete throughout the growing season (Jhala et al. 2014; Kaur et al. 2014). In 2013 no yield was harvested in glyphosate plus cloransulam EPOST fb glyphosate plus lactofen LPOST herbicide program because of an inability to run the combine due to extreme giant ragweed competition, but a yield of 1,184 kg ha^{-1} was recorded in 2014 in the same treatment. Preplant tillage or 2,4-D fb PRE or EPOST fb LPOST treatments resulted in the highest soybean yield (> 2,440 kg ha^{-1}) compared to < 1,800 kg ha^{-1} with PRE fb LPOST herbicide program. Preplant tillage or 2,4-D-only treatments resulted in soybean yield < 720 kg ha^{-1} that clearly demonstrates that preplant tillage or 2,4-D were effective for management of giant ragweed early in the season; however, follow-up application of PRE and/or POST herbicides are needed for effective season-long control of giant ragweed and to avoid yield loss. The contrast statement suggested higher soybean yield with PRE fb LPOST program compared to EPOST fb LPOST program irrespective of preplant treatments, except in 2013, where no differences

were observed in soybean yield between PRE fb LPOST vs EPOST fb LPOST when preceded by preplant treatments (Table 5.3).

This is the first report describing integrated management of glyphosate-resistant giant ragweed in glyphosate-tolerant soybean. Results from this study showed the importance of preplant control of giant ragweed with tillage or 2,4-D fb PRE/POST herbicide treatments. Jhala et al. (2014) and Kaur et al. (2014) reported an effective control of glyphosate-resistant giant ragweed with 2,4-D preplant fb PRE or POST herbicides. While no literature is available on integrated management of giant ragweed with preplant tillage and herbicides, previous studies have reported an effective management of glyphosate-resistant Palmer amaranth with the integrated use of tillage and herbicides (Aulakh et al. 2013; Kelton et al. 2013).

In summary, because giant ragweed is an early emerging weed in Nebraska and exhibits a monophasic emergence pattern (Kaur 2015), preplant tillage is an effective tool for early season management. The alternate approach is application of 2,4-D, particularly in no-till cropping systems. However, continuous use of 2,4-D should be avoided to prevent selection pressure as 2,4-D-resistant common waterhemp has been confirmed in Nebraska in a continuous grass seed production system (Bernards et al. 2012). Therefore, preplant tillage would be a good alternate to include in integrated giant ragweed management programs. The potential limitations of tillage are lack of motivation for the preplant tillage particularly among no-till growers, additional expenses, and weather which is often times not much suitable for early spring tillage.

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Table 5.1. Herbicide treatments, application timing, and rates as well as products used in a field study in Nebraska in 2012 and 2013[a].

Herbicide common name[a]	Timing	Rate	Trade name	Manufacturer	Adjuvant[a,b]
		g ae or ai ha^{-1}			
Sulfentrzone + cloransulam *fb* glyphosate	PRE Late POST	392 870	Authority First Roundup PowerMax	FMC Corporations, Philadelphia, PA 19103; Monsanto Company, 800 North, Lindberg Ave., St. Louis, MO	AMS + COC AMS
Sulfentrzone + cloransulam *fb* glyphosate + fomesafen	PRE Late POST	392 870 + 263	Authority First Roundup PowerMax + Flexstar	FMC Corporations, Philadelphia, PA 19103; Monsanto Company, 800 North, Lindberg Ave., St. Louis, MO + Syngenta Crop Protection, Inc, Greensboro, NC 27419; Bayer Crop Science	AMS + COC AMS + COC
Glyphosate + cloransulam *fb* glyphosate + lactofen	Early POST Late POST	870 + 17.7 870 + 220	Roundup PowerMax + FirstRate Roundup PowerMax + Cobra	Monsanto Company + Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268; Monsanto Company + Valent USA Corporation, Walnut Creek, CA, 94596	AMS + NIS AMS + COC
2,4-D Amine	Preplant	560	2,4-D Amine	Winfield Solutions, LLC, ST PAUL, MN 55164	AMS + NIS
2,4-D Amine *fb* sulfentrzone + cloransulam *fb* glyphosate	Preplant PRE Late POST	560 392 870	2,4-D Amine Authority First Roundup PowerMax	Winfield Solutions FMC Corporations Monsanto Company	AMS + NIS AMS + COC AMS
2,4-D Amine *fb* sulfentrzone + cloransulam *fb* glyphosate + fomesafen	Preplant PRE Late POST	560 392 870 + 263	2,4-D Amine Authority First Roundup PowerMax + Flexstar	Winfield Solutions FMC Corporations Monsanto Company + Syngenta Crop Protection	AMS + NIS AMS + COC AMS + COC
2,4-D Amine *fb* glyphosate + cloransulam *fb* glyphosate + lactofen	Preplant Early POST Late POST	560 870 + 17.7 870 + 220	2,4-D Amine Roundup PowerMax + FirstRate Roundup PowerMax + Cobra	Winfield Solutions Monsanto Company + Dow AgroSciences LLC Monsanto Company + Valent USA Corporation	AMS + NIS AMS + NIS AMS + COC

Sulfentrzone + cloransulam *fb* glyphosate	PRE Late POST	392 870	Authority First Roundup PowerMax	FMC Corporations Monsanto Company	AMS + COC AMS
Sulfentrzone + cloransulam *fb* glyphosate + fomesafen	PRE Late POST	392 870 + 263	Authority First Roundup PowerMax + Flexstar	Monsanto Company + Dow AgroSciences LLC Monsanto Company + Valent USA Corporation	AMS + NIS AMS + COC
Glyphosate + cloransulam *fb* glyphosate + lactofen	Early POST Late POST	870 +17.7 870 + 220	Roundup PowerMax + FirstRate Roundup PowerMax + Cobra	Monsanto Company + Dow AgroSciences LLC Monsanto Company + Valent USA Corporation	AMS + NIS AMS + COC

[a] Abbreviations: ae, acid equivalent; AMS, ammonium sulfate (DSM Chemical North America Inc., Augusta, GA); COC, crop oil concentrate (Agridex, Helena Chemical Co., Collierville, TN); DAPT, days after preplant treatment; DAPRE, days after pre-emergence treatment; DAPOST, days after post-emergence treatment; fb, followed by; MSO, methylated seed oil (Southern Ag Inc., Suwanee, GA); NIS, nonionic surfactant (Induce, Helena Chemical Co.).

[b] AMS at 2% (wt/v), COC or MSO at 1% (v/v), and NIS at 0.25% (v/v) were mixed with herbicides.

Table 5.2. Effect of tillage and herbicides on control of giant ragweed at 7 and 21 d after PRE treatment, 30 and 60 d after early POST treatment, and at harvest in 2013 and 2014 at David City, NE.

Treatment[a]	Application timing	Rate	Giant ragweed control[b,c,d,e]				
			7 DAPRE	21 DAPRE	30 DAEPOST	60 DAEPOST	At harvest
		g ae or ai ha^{-1}	%				
Tillage	Preplant	-	98 a	94 ab	70 c	33 d	10 d
Tillage fb	Preplant		98 a	99 a	99 a	99 a	98 a
sulfentrzone + cloransulam fb	PRE	392					
glyphosate	Late POST	870					
Tillage fb	Preplant		99 a	99 a	99 a	99 a	98 a
sulfentrzone + cloransulam fb	PRE	392					
glyphosate + fomesafen	Late POST	870 + 263					
Tillage fb	Preplant		89 a	92 ab	98 a	98 a	98 a
glyphosate + cloransulam	Early POST	870 + 17.7					
fb glyphosate + lactofen	Late POST	870 + 220					
2,4-D Amine	Preplant	560	95 a	94 ab	67 c	42 d	16 d
2,4-D Amine fb	Preplant	560	98 a	99 a	99 a	99 a	97 a
sulfentrzone + cloransulam fb	PRE	392					
glyphosate	Late POST	870					
2,4-D Amine fb	Preplant	560	97 a	99 a	99 a	98 a	98 a
sulfentrzone + cloransulam fb	PRE	392					
glyphosate + fomesafen	Late POST	870 + 263					
2,4-D Amine fb	Preplant	560	94 a	94 ab	99 a	99 a	98 a
glyphosate + cloransulam fb	Early POST	870 + 17.7					
glyphosate + lactofen	Late POST	870 + 220					
Sulfentrzone + cloransulam fb	PRE	392	74 b	82 b	86 b	81 b	78 b
Glyphosate	Late POST	870					
Sulfentrzone + cloransulam fb	PRE	392	73 b	83 b	84 b	80 b	78 b
glyphosate + fomesafen	Late POST	870 + 263					
Glyphosate + cloransulam	Early POST	870 +17.7	0	0	77 bc	75 c	74 c
fb glyphosate + lactofen	Late POST	870 + 220					
P-value			0.023	0.034	0.041	0.031	0.022
Contrasts							
Preplant fb PRE vs PRE alone			P <0.0001	P <0.0001	-	-	-
Preplant fb PRE fb LPOST vs PRE fb LPOST			-	-	P <0.0001	P <0.0001	P <0.0001
Preplant fb PRE fb LPOST vs Preplant fb EPOST fb LPOST			-	-	P <0.9000	P <0.9872	P <0.9575
PRE fb LPOST vs EPOST fb LPOST			-	-	P <0.0001	P <0.0001	P <0.0001

[a] The experiment was arranged in a split-plot design but to reduce the size of table main (preplant tillage, 2,4-D, no control) and sub-plot (PRE/POST herbicides) treatments were presented in the same column.

[b] Abbreviations: ae, acid equivalent; DAPT, d after preplant treatment; DAPRE, d after PRE; DAEPOST, d after early POST; fb, followed by.

[c] Year-by-treatment interaction was not significant; therefore, data were combined over two years.

[d] Data were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed values.

[e] Means within columns with no common letter(s) are significantly different according to Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

Table 5.3. Effect of tillage and/or herbicide treatments on glyphosate-resistant giant ragweed density, biomass, and soybean yield in a field experiment conducted in 2013 and 2014 at David City, NE.

Treatment[a]	Application timing	Rate	Giant ragweed[b,c,d,e]			Soybean[b,e,f]	
			Density		Biomass reduction	Injury[g]	Yield	
			60 DAEPOST	At harvest	60 DAEPOST	14 DALPOST	2013	2014
		g ae or ai ha^{-1}	No. m^{-2}		%		Kg ha^{-1}	
Nontreated control	-	-	22 a	19 a	0	0	0	0
Tillage	Preplant	-	8 b	8 bc	53 c	0	904 c	656 c
Tillage fb	Preplant		0 d	0 d	100 a	0	2,954 ab	3,071 ab
sulfentrzone + cloransulam fb	PRE	392						
glyphosate	Late POST	870						
Tillage fb	Preplant		1 d	1 d	95 a	12 b	2,881 ab	3,319 a
sulfentrzone + cloransulam fb	PRE	392						
glyphosate + fomesafen	Late POST	870 + 263						
Tillage fb	Preplant		0 d	0 d	99 a	15 ab	2,582 ab	2,445 b
glyphosate + cloransulam fb	Early POST	870 + 17.7						
glyphosate + lactofen	Late POST	870 + 220						
2,4-D amine	Preplant	560	10 b	9 b	45 d	0	1,178 c	716 c
2,4-D amine fb	Preplant	560	1 d	1 d	95 a	0	3,219 a	3,581 a
sulfentrzone + cloransulam fb	PRE	392						
glyphosate	Late POST	870						
2,4-D amine fb	Preplant	560	0 d	0 d	98 a	13 ab	3,492 a	3,301 a
sulfentrzone + cloransulam fb	PRE	392						
glyphosate + fomesafen	Late POST	870 + 263						
2,4-D amine fb	Preplant	560	0 d	1 d	96 a	15 ab	2,862 ab	2,859 ab
glyphosate + cloransulam fb	Early POST	870 + 17.7						
glyphosate + lactofen	Late POST	870 + 220						
Sulfentrzone + cloransulam fb	PRE	392	5 c	3 cd	89 a	0	1,790 bc	1,480 c
Glyphosate	Late POST	870						
Sulfentrzone + cloransulam fb	PRE	392	4 c	2 d	86 ab	12 b	1,355 c	1,196 c
glyphosate + fomesafen	Late POST	870 + 263						
Glyphosate + cloransulam fb	Early POST	870 + 17.7	8 b	9 b	66 bc	16 a	0	1,184 c
glyphosate + lactofen	Late POST	870 + 220						
P-value			<0.0002	<0.0001	0.04	0.008	0.03	0.041
Contrasts								
Preplant fb PRE fb LPOST vs PRE fb LPOST			P <0.0001	P=0.2998	P <0.0001	-	P <0.0001	P <0.0001
Preplant fb PRE fb LPOST vs Preplant fb EPOST fb LPOST			P=0.0010	P=0.3539	P <0.0001	-	P=0.4688	P <0.0001
PRE fb LPOST vs EPOST fb LPOST			P=0.0297	P=0.0173	P <0.0398	-	P <0.0001	P <0.0001

[a] The treatments were arranged in split-plot design but to reduce the size of table main (preplant tillage, 2,4-D) and sub-plot (PRE/POST herbicides) treatments were presented in same column and when PRE/POST herbicides were applied alone, no-preplant control was not mentioned in the table.

[b] Abbreviations: ae, acid equivalent; DAPT, days after preplant treatment; DAPRE, days after PRE; DAEPOST, days after early POST; DALPOST, days after late POST; fb, followed by.

[c] Data were combined over the years for analysis because there was no treatment-by-year interaction.

[d] Data were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

[e] Means within columns with no common letter(s) are significantly different according to the Tukey–Kramer pairwise comparison test at $P \leq 0.05$.

[f] Treatments with zero yield values (nontreated control) were not included in the analysis.

[g] Soybean injury data were collected at 14 d after LPOST; zero values were not included in the analysis.

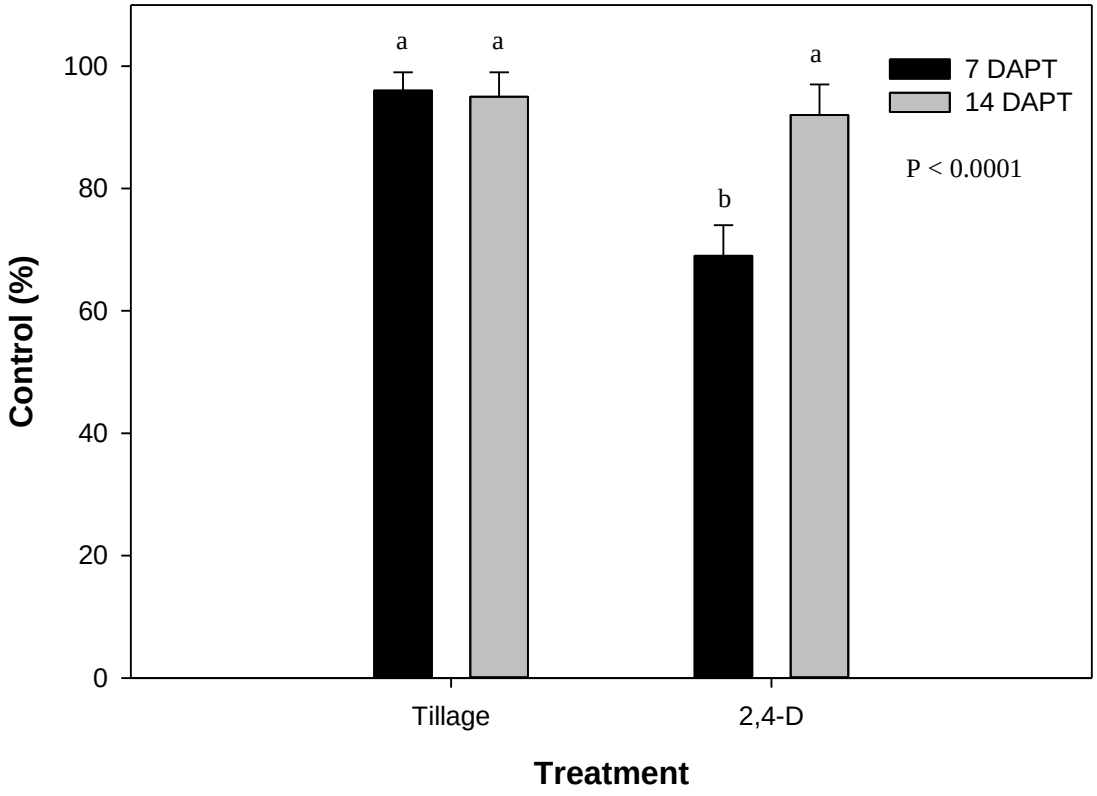

Figure 5.1. Control of glyphosate-resistant giant ragweed at 7 and 14 d after preplant treatment (DAPT) of tillage or 2,4-D in a field experiment conducted at David City, NE in 2013 and 2014. Year-by-treatment interaction was not significant; therefore, data from both years were combined. The bars with no common letter(s) are significantly different according to Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

CHAPTER 6: AN INTEGRATED APPROACH TO CONTROL GLYPHOSATE-RESISTANT GIANT RAGWEED (*Ambrosia trifada*) WITH PREPLANT TILLAGE AND HERBICIDES IN GLYPHOSATE-RESISTANT CORN

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Abstract

Glyphosate-resistant giant ragweed (*Ambrosia trifida*) is a competitive and difficult to control annual broadleaf weed in several agronomic crops in the Midwestern United States and Ontario, Canada. The objectives of this study were to compare treatments for control of glyphosate-resistant giant ragweed with preplant tillage followed by (fb) pre-emergence (PRE) and/or post-emergence (POST) herbicides in glyphosate-resistant corn and to determine the impact of giant ragweed escapes on corn yield. Field experiments were conducted at Clay Center, NE in 2013 and David City, NE in 2014 in grower fields infested with glyphosate-resistant giant ragweed. Preplant tillage resulted in 80 to 85% control compared to no tillage. Preplant tillage fb PRE application of saflufenacil plus dimethenamid-*P* with or without atrazine resulted in 99% control compared to ≤ 86 and 96% control with PRE herbicides alone at 7 and 21 d after PRE (DAPRE), respectively. The PRE fb POST herbicide programs provided ≥ 98% control regardless of preplant tillage. Preplant tillage or POST-only herbicides resulted in 4 to 14 giant ragweed plants m^{-2}, whereas a PRE fb POST program had < 3 plants m^{-2}. Corn yield was greatest

(13,700 to 14, 166 kg ha^{-1}) with the preplant tillage fb PRE and POST herbicide program. The relationship between corn yield and late-season density of giant ragweed escapes showed a 50% corn yield reduction irrespective of control measures when giant ragweed density was 8.44 plants m^{-2}. The combination of preplant tillage with PRE and/or POST herbicides reduced giant ragweed density and biomass accumulation early in the season and provided an integrated approach for effective management.

Introduction

Giant ragweed (*Ambrosia trifida* L.) is a natural colonizer in disturbed areas, a troublesome weed in arable lands, and a threat to human health because of its allergenic pollen—a major cause of hay fever (Abul-Fatih and Bazzaz 1979; Baysinger and Sims 1991). Giant ragweed dominates in common cropland plant communities due to its early emergence, rapid growth rate, high leaf-area index, and ability to tolerate changing environmental factors by adjusting its plant resource utilization response (Abul-Fatih and Bazzaz 1979; Bazzaz and Carlson 1979). These characteristics of giant ragweed result in shading along with rapid consumption of water and nutrients causing intense competition beginning from emergence, leading to significant yield losses (Abul-Fatih and Bazzaz 1979; Barnett and Steckel 2013; Bassett and Crompton 1982).

The commercialization of glyphosate-resistant crops revolutionized weed management by providing excellent weed control and crop safety at a reduced cost (Feng et al. 2010; Nandula 2010). However, over-reliance and continuous use of glyphosate, along with declining trends in the use of other weed management practices (including tillage and soil-applied herbicides) resulted in the evolution of glyphosate-resistant weeds, including giant ragweed (Gianessi 2008; Givens et al. 2009; Young 2006).

Glyphosate-resistant giant ragweed was first reported in Ohio in 2004 (Stachler 2008), and as of 2016 has been confirmed in 12 US states (Heap 2016) and in Ontario, Canada (Sikkema et al. 2009; Vink et al. 2012a). In addition, giant ragweed biotypes resistant to both acetolactate synthase (ALS)-inhibitors and glyphosate have been confirmed in Ohio, Minnesota, and Missouri (Heap 2016).

Lack of diversity in weed management strategies is the main reason for the evolution of herbicide-resistant weeds (Nichols et al. 2009; Talbert and Burgos 2007; Walsh and Powles 2007). Therefore, one of the fundamental considerations for the management of glyphosate-resistant giant ragweed and other herbicide-resistant weeds is the diversification of weed management strategies (Norsworthy et al. 2012; Vencill et al. 2012) using an integrated weed management (IWM) approach. Integrated weed management strategies should consider the use of cultural, mechanical, and chemical control options that are both feasible in specific cropping systems and permitted by socio-economic conditions in order to reduce selection pressure, delay the evolution of new resistant weeds, and ensure effective management of existing herbicide-resistant weeds (Norsworthy et al. 2012; Vencill et al. 2012).

Integrated weed management practices are selected based on the biological and ecological characteristics of the weeds present (Harker and O'Donovan 2013). However, current IWM systems mostly involve chemical plus physical and/or cultural methods, including tillage, cover crops, and crop rotation (Harker and O'Donovan, 2013; Shaw et al. 2012). Tillage is an important tool for managing herbicide-resistant weeds in agronomic crops (Jhala et al. 2014b; Shaw et al. 2012; Shrestha et al. 2006) and there is a need for more judicious, well-timed, and precise use of tillage combined with other

control methods (Beckie and Gill 2006; Shaner and Beckie 2014). The success of tillage, like other weed control methods, is determined by several biological, physical, and environmental factors (Vencill et al. 2012). For example, early emerging weeds such as giant ragweed are easy to control with preplant tillage (Ganie et al. 2016) compared to species that emerge simultaneously with crops and/or have a wide emergence period throughout the season (Hartzler et al. 1999; Wu and Owen 2014).

Giant ragweed competition has been assessed in several agronomic crops, including corn [*Zea mays* (L.)] (Harrison et al. 2001; Williams and Masiunas 2006), soybean [*Glycine max* (L.) Merr.] (Baysinger and Sims 1991), and cotton (*Gossypium hirsutum* L.) (Barnett and Steckel 2013). Harrison et al. (2001) reported that giant ragweed emerging simultaneously with corn resulted in 13 and 60% yield reduction at densities of 1.7 and 13.8 plants 10 m^{-2}, respectively. Similarly, 5% loss of ear mass was reported with an giant ragweed density of 1 plant 25 m^{-2} in sweet corn (Williams and Masiunas 2006). Giant ragweed is even more competitive in soybean, with 1 plant m^{-2} causing 45 to 77% yield loss (Baysinger and Sims 1991; Webster et al. 1994). However, there is no literature available describing the impact of giant ragweed that escapes weed control on crop yield, information that is necessary due to rising concerns about the frequently occurring late-season giant ragweed escapes in the eastern Corn Belt (Williams and Masiunas 2006). Previously, Johnson et al. (2004) reported giant ragweed as a predominant late-season weed in Indiana soybean fields rotated with corn. A recent survey in Wisconsin also reported giant ragweed among the most common late-season escape weed species in glyphosate dependent corn-soybean cropping systems (Recker et al. 2015). Most common causes of weed escapes have been reported by Bagavathiannan

and Norsworthy (2012); however, the main reason for variable control of giant ragweed with POST herbicides is emergence from different soil depths resulting in variable plant sizes and leaf area. Small plants are sheltered by larger giant ragweed plants, resulting in either zero or partial spray coverage of the POST herbicide, usually resulting in variable control (personal observation). In addition, early-season management influences the size of giant ragweed plants at the time of POST herbicide treatments. Loux et al. (2015) reported that at the time of POST herbicide application, 63% of giant ragweed plants were > 15 cm tall and 31% were > 30 cm in the absence of preplant treatment, whereas 95 and 99% of plants were < 15 cm tall with preplant alone and preplant fb PRE herbicide programs, respectively.

A recent study in Nebraska confirmed that early-spring tillage had no effect on the emergence pattern of giant ragweed (Kaur et al. 2016). The study reported here was initiated based on the hypothesis that preplant tillage would provide effective early-season control of giant ragweed to allow corn planting in a weed-free environment and improve the efficacy of PRE and POST herbicides. It was further hypothesized that giant ragweed escapes under the management programs evaluated in this study will have a direct impact on corn yield. The objectives of this study were (1) To evaluate the efficacy of integrated management of glyphosate-resistant giant ragweed with preplant tillage followed by (fb) PRE and/or POST herbicides vs PRE and/or POST herbicides alone, and (2) To determine the relationship between the density of giant ragweed escapes under the evaluated management programs and corn yield.

Materials and Methods

Field experiments were conducted at Clay Center (40.52°N, 98.05°W) and David City (41.25°N, 97.13°W), Nebraska in 2013 and 2014, respectively, in grower fields infested with glyphosate-resistant giant ragweed. Giant ragweed biotypes from these sites were confirmed resistant to glyphosate in 2011, with the level of resistance ranging from 9× to 14× [where × is the labeled rate of glyphosate (1,050 g ae ha^{-1})] compared to susceptible biotypes (Rana et al. 2013). The density of glyphosate-resistant giant ragweed at these sites varied from 18 to 32 plants m^{-2}. The soil type at Clay Center was silt loam with 17% sand, 58% silt, 25% clay, 2.5% organic matter, and a pH of 6.5. The soil type at David City was silty clay loam with 18% sand, 50% silt, 32% clay, 2.1% organic matter, and a pH of 5.4. The experiment was arranged in a split-plot design with four replications, where the main-plot was preplant tillage or no preplant tillage and the sub-plot was PRE and/or POST herbicide treatments for a total of 16 treatment combinations (Table 6.1). Treatment with no preplant tillage or herbicide application served as the nontreated control and tillage alone as a no herbicide control. Application rates of herbicides were based on their labeled rates in corn. Glyphosate-resistant corn seeds (Cv. "Pioneer 1151R" in 2013 and "Mycogen 2V709" in 2014) were planted on May 16, 2013 and May 17, 2014. The seeds were planted 3 cm deep at a density of 79,000 seeds ha^{-1}. Individual plots were 3 m wide and 9 m long with 4 corn rows spaced 76 cm apart.

Preplant tillage was accomplished using a tandem disk on May 2, 2013 and May 3, 2014. Herbicide treatments were applied as PRE (May 16, 2013 and May 17, 2014) and POST (June 8, 2013 and June 9, 2014) on 6 to 15 cm tall (2 to 6 leaf stage) giant ragweed plants. Herbicides were applied with a CO_2-pressurized backpack sprayer

calibrated to deliver 140 L ha^{-1} at 276 kPa equipped with a four-nozzle boom fitted with AIXR 110015 flat-fan nozzles (TeeJet, Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60189). The experimental locations were under rain-fed/non-irrigated conditions during both years.

Visual control of giant ragweed was assessed using a scale of 0 to 100% (0 being no control and 100 being complete control) at 7 and 14 d after preplant treatments (DAPT); 7, 14, and 21 d after PRE herbicide treatments; 30 and 60 d after POST herbicide treatments, and at harvest. Herbicide injury on corn was recorded using a scale of 0 to 100% (0 being no injury and 100 being plant death) at 14 and 21 d after PRE and POST herbicide treatments. Giant ragweed density was recorded from three randomly placed 0.25 m^2 quadrats per plot at 21 d after PRE herbicide treatments, 60 d after POST herbicide treatments, and 2 week before corn harvest. Glyphosate-resistant giant ragweed biomass was assessed from three randomly placed 0.25 m^2 quadrats per plot at 60 d after POST herbicide application. Giant ragweed plants that survived herbicide treatment were cut at the stem base close to the soil surface, placed in paper bags, dried in an oven for 70 h at 55 C, and weighed (g). Two center rows of corn were harvested using a plot combine and yields were adjusted to 15% moisture content (Harrison et al. 2001). Giant ragweed biomass data were converted into percent biomass reduction compared to the nontreated control (Wortman 2014) as:

$$\text{Percent biomass reduction} = \left[{}^{(\overline{C} - B)}\!/_{\overline{C}} \right]^{\bullet} 100 \qquad [1]$$

where $\overline{C}$ is the mean biomass of the four nontreated control plots and B is the biomass of an individual treated experimental unit.

Statistical Analysis. Data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS version 9.3 (SAS Institute Inc, Cary, NC). Since sub-plot treatments (PRE/POST herbicides) were not applied until 14 DAPT, least square means for the visual control estimates of giant ragweed at 7 and 14 DAPT were analyzed as a randomized complete block design with preplant control methods (preplant tillage or no preplant tillage), year and their interactions considered as fixed effects, and replication as a random effect in the model. All other data were analyzed as split-plot design with preplant control methods, PRE and/or POST herbicides, year and their interactions considered as fixed effects and the replications as a random effect in the model. The treatment combinations with zero response variables were not included in the data analyses. Before analyses, data were tested for normality of residuals using the PROC UNIVARIATE procedure in SAS. Visual estimates of giant ragweed control, density, and biomass data were arcsine square-root transformed before analysis; however, back-transformed data are presented with mean separation based on transformed data. When the ANOVA indicated treatment effects were significant, means were separated at $P \leq 0.05$ using Tukey-Kramer's pairwise comparison test. Preplanned single degree-of-freedom contrast statements were used to compare management programs by testing specific hypotheses, including tillage fb PRE vs PRE, tillage fb POST vs POST, and tillage fb PRE fb POST vs PRE fb POST.

A two-parameter hyperbolic regression model (Equation 2) was fitted to determine the relationship between corn yield and density of giant ragweed escapes under the management approaches evaluated in this study (Barnett and Steckel 2013) using R

software (R statistical software, R Foundation for Statistical Computing, Vienna, Austria).

$$y = [\frac{ab}{b+x}] \qquad [2]$$

where y is corn yield (kg ha^{-1}), a is the upper asymptote or estimate of maximum yield, b is the estimate of giant ragweed density (plants m^{-2}) that causes 50% reduction in corn yield, and × is giant ragweed density (plants m^{-2}).

Results and Discussion

Year-by-treatment interactions for giant ragweed visual estimations of control, density, biomass, corn injury, and yield were not significant; therefore, data were combined over years. However, the interaction between main plot treatments (preplant tillage and no-preplant control) and sub-plot treatments (PRE/POST herbicides) was significant ($P < 0.05$) for all variables; therefore, mean separation for the simple effects are presented.

Giant Ragweed Control. Preplant tillage resulted in 80 to 85% control of glyphosate-resistant giant ragweed at 7 and 14 d after preplant tillage (DAPT) compared to no preplant tillage (data not shown). However, giant ragweed control following preplant tillage without PRE or POST herbicides declined due to new emergence or regrowth of partially controlled giant ragweed. For example, giant ragweed control with tillage without follow-up PRE or POST herbicides declined to 55 and 46% at 30 and 60 d after POST herbicide treatment (DAPOST), respectively (Table 6.2). Similarly, Ganie et al. (2016) reported > 90% early-season control of giant ragweed with preplant tillage, though the maintenance of effective control was dependent on follow-up applications of PRE and/or POST herbicide treatments.

Contrast analysis was performed to test the hypothesis that tillage followed by PRE herbicides would result in greater giant ragweed control compared with PRE herbicides without tillage. The results showed that preplant tillage fb PRE herbicides provided 99% giant ragweed control compared to 85% control with PRE-only herbicides at 7 DAPRE (Table 6.3). Similarly, preplant tillage fb PRE application of saflufenacil plus dimethenamid-*P* with or without atrazine resulted in 99% control of giant ragweed at 21 DAPRE treatment (Table 6.2). However, without preplant tillage, the same treatment resulted in 95 to 96% control because early emerged giant ragweed plants that had already been established were not controlled by PRE herbicides (Table 6.2). Previous studies reported ≥ 87% control of glyphosate-resistant giant ragweed with a tank-mixture of glyphosate, saflufenacil, and dimethenamid-*P* (Belfry and Sikkema 2015) and 63% control with saflufenacil plus dimethenamid-*P* (Soltani et al. 2011) at 28 DAPRE. Recently, Ganie et al. (2016) reported > 96% control of glyphosate-reistant giant ragweed in soybean with preplant tillage or 2,4-D fb sulfentrazon plus cloransulam PRE compared to ≤ 86% control with the same herbicide treatments without preplant tillage or 2,4 D at 21 DAPRE. Results of this study suggested the importance of preplant tillage to supplement PRE herbicides for effective early season management of giant ragweed in corn.

Contrast analysis was performed to test the hypothesis that POST application of 2,4-D or halosulfuron plus dicamba plus glyphosate preceded by preplant tillage would provide better giant ragweed control than the same treatment without tillage. Tillage fb POST herbicides resulted in 95 to 97% control of giant ragweed compared to 90 to 95% control with POST-only herbicide programs at 30 DAPOST, 60 DAPOST, and at harvest

(Table 6.3). Although both tillage fb POST and POST-only herbicide programs provided > 90% control, tillage fb POST is more desirable because of its potential to allow corn planting under reduced weed pressure and less giant ragweed competition during corn emergence.

Contrast statements to test the hypothesis that giant ragweed control would be greater in tillage fb PRE fb POST herbicide programs compared to PRE fb POST herbicide programs were not significant (Table 6.3). Both of these management programs including herbicide mixtures such as saflufenacil plus dimethenamid-*P* with or without atrazine as PRE and glyphosate or halosulfuron plus dicamba plus glyphosate or tembotrione plus atrazine applied POST resulted in 99% control of glyphosate-resistant giant ragweed at 30, 60 DAPOST, and at harvest, regardless of preplant tillage (Tables 6.2 and 6.3). Results suggested that herbicide mixtures based on different biochemical sites-of-action applied PRE fb POST provided effective season-long control of giant ragweed. However, tillage is favorable to diversify the management approach, reduce dependence on herbicides, and mitigate herbicide selection pressure for resistance by exposing fewer plants to herbicide(s) (Gressel and Levy 2006; Norsworthy et al. 2012).

Herbicide programs exist for effective control of glyphosate-resistant giant ragweed in corn. However, diversity in management approaches is needed for a true integrated weed management program (Harker and O'Donovan 2013). Results of this study suggested that preplant tillage provided effective (> 80%) early-season control of giant ragweed emerged at the time of preplant tillage and allowed corn to be planted under reduced giant ragweed pressure (< 20%). Wilson (1993) reported 86% reduction in weed pressure with preplant tillage compared to no-tillage, and observed broad-spectrum

weed control by integrating preplant tillage with herbicides compared to tillage or herbicides applied alone. Similarly, the inclusion of tillage to supplement herbicides for the successful management of glyphosate-resistant Palmer amaranth [*Amaranthus palmeri* S. (Wats.)] has been documented in several studies in the southern United States (Aulakh et al. 2012; 2013; Kelton et al. 2013).

Giant Ragweed Density and Biomass. The results of giant ragweed visual control estimates were reflected in giant ragweed density and biomass (Table 6.4 and 6.5). The greatest giant ragweed density at harvest was observed in the nontreated control (≥ 26 plants m^{-2}) fb preplant tillage alone (≥ 12 plants m^{-2}). Contrast analysis between tillage fb POST herbicide and POST-only herbicide program was significant ($P < 0.0007$) at 60 DAPOST; however, tillage fb PRE fb POST herbicide vs PRE fb POST herbicide was not significant ($P = 0.8193$). PRE fb POST herbicide programs reduced giant ragweed density to < 2.0 plants m^{-2} irrespective of preplant tillage. However, density varied from 2 to 3 plants m^{-2} with preplant tillage fb POST herbicides, and 2 to 5 plants m^{-2} with a POST-only herbicide program (Table 6.4). Similarly, Riley et al. (2014) reported the greatest reduction in giant ragweed density (< 6 plants m^{-2}) with a preplant application of glyphosate plus 2,4-D, dicamba, or saflufenacil fb glyphosate alone or glyphosate plus fomesafen or cloransulam or chlorimuron in glyphosate-resistant soybean.

Contrast analysis to test the hypothesis that reduction in giant ragweed biomass would be greater with preplant tillage fb PRE fb POST compared to a PRE fb POST herbicide program was not significant ($P = 0.2620$). PRE fb POST herbicide programs resulted in > 98% biomass reduction of giant ragweed at 60 DAPOST irrespective of preplant tillage (Table 6.4). Similarly, previous studies reported 75 to 100% reduction in

giant ragweed biomass with preplant or PRE fb POST herbicide programs (Jhala et al. 2014a; Kaur et al. 2014; Vink et al. 2012b). Contrasts between tillage fb POST and a POST-only herbicide program was significant (P = 0.04), and results indicated that tillage fb POST herbicide programs reduced giant ragweed biomass by 92% compared to 85% for the POST-only herbicide programs (Table 6.4). However, 90 to 94% reduction in giant ragweed biomass was observed among POST-only treatments irrespective of preplant tillage, except for a 77% biomass reduction with 2,4-D applied POST without preplant tillage (Table 6.5). In contrast, Robinson et al. (2012) reported 96 to 99% reduction in giant ragweed biomass with 2,4-D applied POST. Biomass reduction with only preplant tillage was 24%, indicating the failure of preplant tillage alone to control giant ragweed later in the season (Table 6.5). Similarly, Jhala et al. (2014a) and Kaur et al. (2014) reported that preplant herbicide application alone was not sufficient to achieve season-long control of giant ragweed and that a follow-up application of a PRE or POST herbicide was needed.

Corn Injury and Yield. Corn injury was 2 to 4% at 14 d after PRE herbicide treatments; however, injuries were transient and not visible at 30 d after treatment (Table 6.5). Contrast analyses to test the hypothesis that tillage fb POST and tillage fb PRE fb POST herbicide programs would result in greater corn yield compared to POST-only and PRE fb POST herbicide programs, respectively, were significant (P < 0.0030). Tillage fb POST herbicides resulted in average corn yield of 12,627 kg ha^{-1} compared to 8,491 kg ha^{-1} with POST-only program (Table 6.4). Similarly, tillage fb PRE fb POST herbicides resulted in average corn yield of 13,714 kg ha^{-1} compared to 12,387 kg ha^{-1} with PRE fb POST herbicide program, indicating the importance of preplant tillage for control of giant

ragweed (Table 6.4). The POST-only application of 2,4-D or halosulfuron plus dicamba plus glyphosate resulted in corn yields ranging from 7,888 to 9,093 kg ha^{-1}, which was greater than the yield with only preplant tillage (4,597 kg ha^{-1}) (Table 6.5). Results of this study highlight the importance of early season control of giant ragweed using preplant tillage to reduce competition with corn early in the season. Additionally, follow-up PRE and/or POST herbicides would be needed for effective season-long control of giant ragweed and to achieve greater corn yield.

Impact of Giant Ragweed Escapes on Corn Yield. Size and density of giant ragweed plants varied at the time of POST herbicide application (21 DAPRE), depending on prior control measures. For example, preplant tillage alone resulted in < 50% reduction in giant ragweed density compared to ≥ 90% reduction with preplant tillage fb PRE herbicides (Table 6.5). Regrowth of partially controlled giant ragweed plants and new emergence resulted in a mixed stand of plants varying from 8 to 15 cm in height at the time of POST herbicide application. Similarly, Loux et al. (2015) reported that without early season control, 63% of giant ragweed plants were > 15 cm tall and 31% of plants were > 30 cm tall at the time of POST herbicide application compared to preplant fb PRE herbicide programs, where 99% of plants were < 15 cm tall. Since most of the escaped giant ragweed plants emerged early (before POST herbicide application) and continued to grow later in the season, they are more likely to interfere with corn growth and impact yield compared with plants controlled by preplant tillage and in-crop herbicides. Therefore, corn yield and the density of giant ragweed escapes under different management approaches were correlated and this relationship was explained by a two-parameter hyperbolic regression model. The estimated parameters of the model for yield

vs giant ragweed density at 60 DAPOST were $y = [\frac{14{,}385 \times 8.44}{8.44+x}]$ with a root mean square error (RMSE) of 3,273; where y represents yield (kg ha^{-1}) and × represents giant ragweed density (plants m^{-2}). The model predicted that giant ragweed density of 8.44 plants m^{-2} allowed to compete up to 60 DAPOST herbicide application has the potential to cause 50% reduction in corn yield (Figure 6.1). Rapid growth rate, larger leaf size, and the ability to grow taller than the crop enable giant ragweed to compete with crops even at lower densities and often require a second POST herbicide application for effective control and to prevent seed production (Anonymous 2015; Loux et al. 2015). Earlier studies have reported giant ragweed as the most competitive weed in corn, soybean, and cotton (Baysinger and Sims 1991; Barnett and Steckel 2013; Harrison et al. 2001). Barnett and Steckel (2013) reported 50% reduction in cotton lint yield with 0.26 plants m^{-1} row. Similarly, Harrison et al. (2001) reported 13.6% yield loss in corn with 1 giant ragweed plant 10 m^{-2}. Additionally, they also reported a reduction in giant ragweed interference with a 4 wk delay in emergence compared to corn.

Results of this study indicate that preplant tillage provides effective early season control of giant ragweed and supplements follow-up herbicides, but the use of PRE and/or POST herbicides or herbicide mixtures with different sites-of-action is indispensable, since giant ragweed escapes can result in yield loss. Earlier we reported similar results in soybean where preplant tillage or 2,4-D fb PRE and/or POST herbicides provided effective (> 95%) giant ragweed control compared to a PRE fb POST herbicide program (Ganie et al. 2016). Thus, preplant tillage can be a potential tool for the integrated management of glyphosate-resistant giant ragweed in corn-soybean cropping systems. Future studies should consider integrating herbicides with additional non-

chemical control strategies, including cover crops, harvest weed seed destruction, and narrow-row planting to reduce selection pressure while providing an effective integrated resistance management strategy.

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Table 6.1. Herbicide treatments, application timing, rates, and products used in a field study for control of glyphosate-resistant giant ragweed in glyphosate-resistant corn in Nebraska in 2013 and 2014.[a]

Herbicide common name	Timing	Rate	Trade name	Manufacturer	Adjuvant [b]
		g ae or ai ha^{-1}			
2,4-D amine	POST	534	2,4-D amine	Winfield Solutions, LLC, St Paul, MN 55164; www.winfield.com	AMS + NIS
Saflufenacil + dimethenamid-*P fb*	PRE	780	Verdict	BASF Corporation, 26 Davis, Research Triangle Park, NC;	AMS +
glyphosate	POST	1,260	Roundup PowerMax	www.basf.com	MSO
				Monsanto Company, 800 North, Lindberg Ave., St. Louis, MO;	AMS
				www.monsanto.com	
Atrazine + saflufenacil +	PRE	2470 + 780	Aatrex+ Verdict	Syngenta Crop Protection, Inc, Greensboro, NC 27419 + BASF	AMS +
dimethenamid-*P fb* glyphosate	POST	1260	Roundup PowerMax	Corporation; Monsanto Company	MSO
					AMS
Saflufenacil + dimethenamid-*P fb*	PRE	780	Verdict	BASF Corporation	AMS +
2,4-D amine + glyphosate	POST	534 + 1,260	2,4-D amine +	Winfield Solutions + Monsanto Company	MSO
			Roundup PowerMax		AMS
Halosulfuron + dicamba + glyphosate	POST	380 + 1,260	Yukon + Roundup	Gowan Company, Yuma, AZ 85366; www.gowanco.com +	AMS + NIS
			PowerMax	Monsanto Company	
Saflufenacil + dimethenamid-*P fb*	PRE	780	Verdict	BASF Corporation	AMS +
halosulfuron + dicamba +	POST	380 + 1,260	Yukon + Roundup	Gowan Company + Monsanto Company	MSO
glyphosate			PowerMax		AMS + NIS
Saflufenacil + dimethenamid-*P fb*	PRE	780	Verdict	BASF Corporation	AMS +
tembotrione + atrazine	POST	92 + 560	Laudis + Aatrex	Bayer Crop Science, Research Triangle	MSO
				Park, NC 27709; www.cropscience.bayer.com + Syngenta Crop	
				Protection	

[a] Abbreviations: ae, acid equivalent; fb, followed; AMS (ammonium sulfate, DSM Chemicals North America Inc., Augusta, GA); COC (crop oil concentrate, Agridex, Helena Chemical Co., Collierville, TN); MSO (methylated seed oil, Southern Ag Inc., Suwanee, GA); NIS (nonionic surfactant, Induce, Helena Chemical Co., Collierville, TN).

[b] AMS at 2% (wt/v), COC or MSO at 1% (v/v), and NIS at 0.25% (v/v) were mixed with herbicides.

Table 6.2. Effect of tillage and/or herbicides on control of glyphosate-resistant giant ragweed at 7 and 21 DAPRE, 30 and 60 DAPOST treatment, and at harvest in glyphosate-resistant corn in 2013 and 2014 at Clay Centre and David City, NE, respectively.[a, b, c]

Treatment	Application timing	Rate	Giant ragweed control after PRE and POST treatments [d, e]				
			7 DAPRE	21 DAPRE	30 DAPOST	60 DAPOST	At harvest
		g ae or ai ha^{-1}					
			%				
Tillage	Preplant	-	75 c	66 c	55 c	45 c	36 c
Tillage *fb*	Preplant	-	73 c	64 c	95 ab	97 ab	97 a
2,4-D amine	POST	534					
Tillage *fb*	Preplant	-	99 a	99 a	99 a	99 a	99 a
saflufenacil + dimethenamid-*P fb*	PRE	780					
glyphosate	POST	1,260					
Tillage *fb*	Preplant	-	99 a	99 a	99 a	99 a	98 a
atrazine + saflufenacil + dimethenamid-*P fb*	PRE	2,470 + 780					
glyphosate	POST	1,260					
Tillage *fb*	Preplant	-	99 a	99 a	99 a	99 a	99 a
saflufenacil + dimethenamid-*P fb*	PRE	780					
2,4-D amine + glyphosate	POST	534 + 1,260					
Tillage *fb*	Preplant	-	73 c	66 c	96 ab	97 ab	96 ab
halosulfuron + dicamba + glyphosate	POST	380 + 1,260					
Tillage *fb*	Preplant	-	99 a	99 a	99 a	99 a	99 a
saflufenacil + dimethenamid-*P fb*	PRE	780					
halosulfuron + dicamba + glyphosate	POST	380 + 1,260					
Tillage *fb*	Preplant	-	99 a	99 a	99 a	98 ab	99 a
saflufenacil + dimethenamid-*P fb*	PRE	780					
tembotrione + atrazine	POST	92 + 560					
2,4-D amine	POST	534	0	0	91 b	92 b	90 b
Saflufenacil + dimethenamid-*P fb*	PRE	780	86 b	96 b	99 a	99 a	99 a
Glyphosate	POST	1,540					
Atrazine + saflufenacil + dimethenamid-*P fb*	PRE	2,470 + 780	84 b	95 b	99 a	99 a	99 a
Glyphosate	POST	1,260					
Saflufenacil + dimethenamid-*P fb*	PRE	780	85 b	96 b	99 a	99 a	99 a
2,4-D amine + glyphosate	POST	534 + 1,260					
Halosulfuron + dicamba + glyphosate	POST	380 + 1,260	0	0	95 ab	93 ab	90 b
Saflufenacil + dimethenamid-*P fb*	PRE	780	85 b	96 b	99 a	99 a	99 a
halosulfuron + dicamba + glyphosate	POST	380 + 1,260					
Saflufenacil + dimethenamid-*P fb*	PRE	780	85 b	95 b	99 a	99 a	99 a
tembotrione + atrazine	POST	92 + 560					
P-value			0.04	<0.0001	0.0142	<0.0001	<0.0001

[a] Abbreviations: ae, acid equivalent; DAPRE, days after PRE; DAPOST, days after POST; fb, followed by.

[b] The treatments were arranged in a split-plot design but to reduce the size of the table, main-plot (tillage/no tillage) and sub-plot (PRE/POST herbicides) treatments are presented in the same column and when PRE/POST herbicides were applied alone, no-preplant control was mentioned in the table. Additionally, nontreated control treatment with zero response variables was not included in analysis or in this table.

[c] Year-by-treatment interaction was not significant; therefore, data for both years were combined.

[d] Data were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

[e] Means within columns with no common letter(s) are significantly different according to the Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

Table 6.3. Contrast means for control of glyphosate-resistant giant ragweed in corn in different management programs in field experiments conducted in Nebraska in 2013 and 2014.[a, b]

Treatment	Giant ragweed control [c]				
	7 DAPRE	21 DAPRE	30 DAPOST	60 DAPOST	At harvest
	%				
Tillage fb PRE	99	99	-	-	-
PRE	85	96	-	-	-
Tillage fb POST	-	-	96	97	97
POST	-	-	93	92	90
Tillage fb PRE fb POST	-	-	99	99	99
PRE fb POST	-	-	99	99	99
Tillage fb PRE vs PRE only	P<0.0001	P<0.0001	-	-	-
Tillage fb POST vs POST only	-	-	P=0.0139	P=0.0002	P <0.0001
Tillage fb PRE fb POST vs PRE fb POST	-	-	P=0.5206	P=0.4986	P=0.3281

[a] Abbreviations: DAPRE, days after PRE; DAPOST, days after POST; fb, followed by.
[b] Year-by-treatment interaction was not significant; therefore, data for both years were combined.
[c] P-values are based on single-degree of freedom contrast analysis.

Table 6.4. Contrast means for density and biomass reduction of glyphosate-resistant giant ragweed in corn and corn seed yield under different management programs in field experiments conducted in Nebraska in 2013 and 2014.[a, b, c]

Treatment	Giant ragweed				Corn yield
	Density			Biomass reduction	
	21 DAPRE	60 DAPOST	At harvest		
	No. m^{-2}			%	Kg ha^{-1}
Tillage fb PRE	1	-	-	-	-
PRE	2	-	-	-	-
Tillage fb POST	-	3	2	92	12,627
POST	-	5	2	85	8,491
Tillage fb PRE fb POST	-	1	0	99	13,714
PRE fb POST	-	1	0	99	12,387
Tillage fb PRE vs PRE only	P=0.0238		-		
Tillage fb POST vs POST only	-	P=0.0007	P=0.8192	P =0.0400	P <0.0001
Tillage fb PRE fb POST vs PRE fb POST	-	P=0.8193	P=1.000	P=0.2620	P <0.0030

[a] Abbreviations: DAPRE, days after PRE; DAPOST, days after POST; fb, followed by.

[b] Year-by-treatment interaction was not significant; therefore, data for both years were combined.

[c] P-values are based on single-degree of freedom contrast analysis.

Table 6.5. Effect of different management programs on glyphosate-resistant giant ragweed density, biomass reduction, corn injury, and seed yield in field experiments conducted in 2013 and 2014 at Clay Centre and David City, NE, respectively.[a, b, c, d]

Herbicide	Application timing	Rate	Giant ragweed [e, f]				Corn [e]	
			Density			Biomass reduction	Injury	Yield
			21 DAPRE	60 DAPOST	At harvest	60 DAPOST	14 DAPRE	
		g ae or ai ha^{-1}	No. m^{-2}			%	%	Kg ha^{-1}
Nontreated control		-	31 a	29 a	26 a	-	0	0
Tillage	Preplant	-	14 b	14 b	12 b	24 d	0	4,597 d
Tillage *fb*	Preplant	-	13 b	3 cd	2 c	90 b	0	12,799 ab
2,4-D amine	POST	534						
Tillage *fb*	Preplant	-	1 c	1 d	0 c	99 a	3 a	13,435 a
saflufenacil + dimethenamid-*P fb*	PRE	780						
glyphosate	POST	1,260						
Tillage *fb*	Preplant	-	1 c	1 d	0 c	99 a	3 a	13,822 a
atrazine + saflufenacil + dimethenamid-*P fb*	PRE	2,470 + 780 -						
glyphosate	POST	1,260						
Tillage *fb*	Preplant	-	1 c	1 d	0 c	99 a	3 a	14,028 a
saflufenacil + dimethenamid-*P fb*	PRE	780						
2,4-D amine + glyphosate	POST	534 + 1,260						
Tillage *fb*	Preplant	-	10 b	2 d	2 c	94 ab	0	12,454 ab
halosulfuron + dicamba + glyphosate	POST	380 + 1,260						
Tillage *fb*	Preplant	-	1 c	1 d	0 c	99 a	2 a	13,121 a
saflufenacil + dimethenamid-*P fb*	PRE	780						
halosulfuron + dicamba + glyphosate	POST	380 + 1,260						
Tillage *fb*	Preplant	-	1 c	1 d	0 c	99 a	3 a	14,166 a
saflufenacil + dimethenamid-*P fb*	PRE	780						
tembotrione + atrazine	POST	92 + 560						
2,4-D amine	POST	534	27 a	6 c	2 c	77 c	0	7,888 c
Saflufenacil + dimethenamid-*P fb*	PRE	780	2 c	1 d	0 c	99 a	3 a	12,535 ab
Glyphosate	POST	1,260						
Atrazine + saflufenacil + dimethenamid-*P fb*	PRE	2,470 + 780	2 c	1 d	0 c	97 ab	2 a	12,998 ab
Glyphosate	POST	1,260						
Saflufenacil + dimethenamid-*P fb*	PRE	780	2 c	1 d	0 c	99 a	3 a	11,960 ab
2,4-D amine + glyphosate	POST	534 + 1,260						
Halosulfuron + dicamba + glyphosate	POST	380 + 1,260	26 a	4 cd	2 c	92 b	0	9,093 bc
Saflufenacil + dimethenamid-*P fb*	PRE	780	2 c	1 d	0 c	99 a	3 a	12,198 ab
halosulfuron + dicamba + glyphosate	POST	380 + 1,260						
Saflufenacil + dimethenamid-*P fb*	PRE	780	3 c	1 d	0 c	99 a	4 a	12,245 ab
tembotrione + atrazine	POST	92 + 560						
P-value			<0.0001	0.0002	<0.0001	0.044	0.990	0.0302

[a] Abbreviations: ae, acid equivalent; DAPRE, days after PRE; DAPOST, days after POST; fb, followed by.

[b] Treatments were arranged in a split-plot design but to reduce the size of the table main (tillage/no tillage) and sub-plot (PRE/POST herbicides) treatments were presented in same column and when PRE/POST herbicides were applied alone, no-preplant control was mentioned in the table.

[c] Treatments with 0% corn injury and no corn yield (0 kg ha^{-1}) were not included in the analysis.

[d] Year-by-treatment interaction was not significant; therefore, data for both years were combined.

[e] Giant ragweed density and biomass data presented were collected at 60 DAPOST, and the data were arc-sine square-root transformed before analysis; however, data presented are the means of actual values for comparison based on interpretation from the transformed data.

[f] Means within columns with no common letter(s) are significantly different according to the Tukey–Kramer's pairwise comparison test at $P \leq 0.05$.

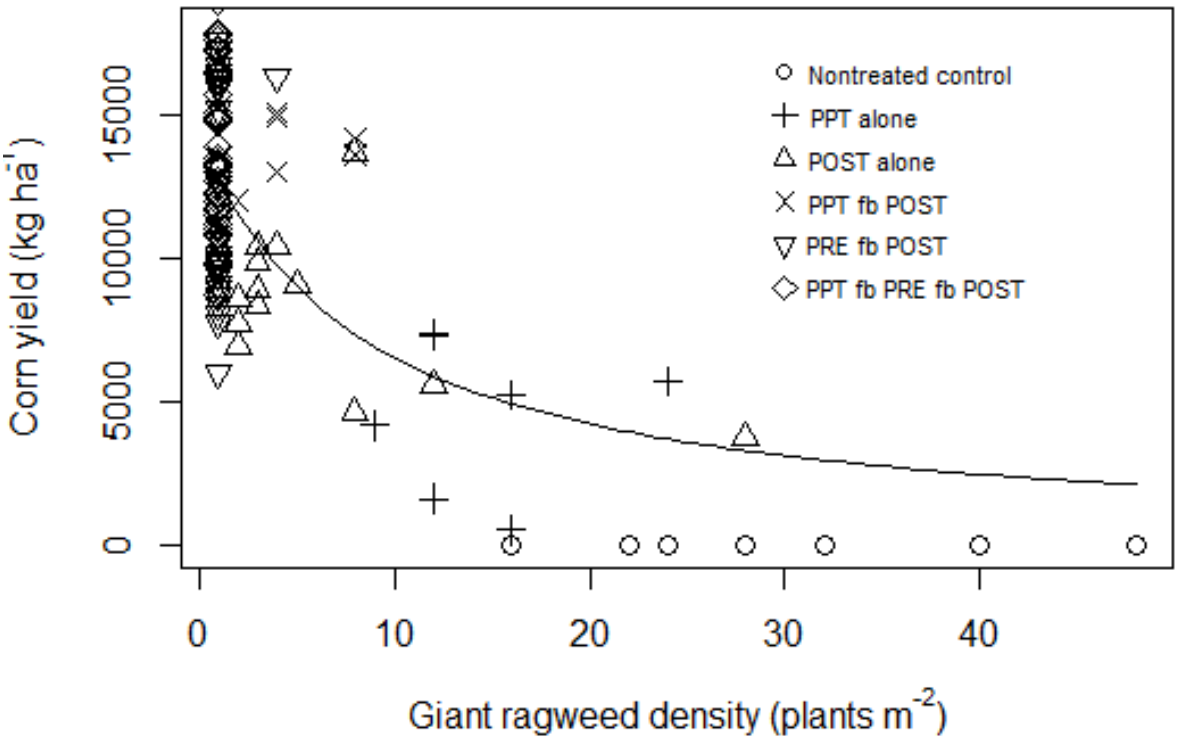

Figure 1. Corn yield relative to density of glyphosate-resistant giant ragweed. The fitted line is calculated from the two-parameter hyperbolic model, $y = [\frac{ab}{b+x}]$, where y is corn yield (kg ha^{-1}), a is the upper asymptote or estimate of maximum yield, b is the estimate of giant ragweed density (plants m^{-2}) which causes 50% reduction in corn yield, and × is the giant ragweed density (plants m^{-2}). The estimated parameters were $y = [\frac{14{,}385 \times 8.44}{8.44+x}]$ and root mean square error (RMSE) of 3,273. Abbreviations: "fb" = followed by, "PPT" = preplant tillage, "POST" = post emergence, "PRE" = pre-emergence.